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EFFECT OF EXPOSURE TO COLD ON THE DISTRIBUTION OF BLOOD FLOW IN SHEEP

by

© A. L. Schaefer

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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IN
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Department of Animal Science

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECT OF EXPOSURE TO COLD ON THE DISTRIBUTION OF BLOOD FLOW IN SHEEP submitted by A. L. Schaefer in partial fulfilment of the requirements for the degree of Master of Science in Animal Physiology.

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Supervisor

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Date January 30 1979

DEDICATION

I wish to dedicate this thesis to my wife Jane, without whose encouragement, assistance, support, and patience, my ability to carry out this work would have greatly suffered.

ABSTRACT

The effects of acute and chronic cold exposure on the tissue distribution of cardiac output and blood flow were measured in twelve, six-month-old wether sheep. Prior to the measurement of blood flow the animals were randomly allocated into one of three treatment groups and exposed to either; (1)near thermoneutral conditions, designated the control group (CON); (2) 12 hr of cold, designated the acute cold group (AC); (3) 10-12 weeks of cold, designated the chronic cold group (CC). The control group as well as both cold treatment groups prior to cold exposure were maintained at temperatures of 18±4.5 C dry bulb (db), 11±2.8 C wet bulb (wb). Both the AC and CC groups were exposed to 3±1.4 C db, 0±0.8 C wb during their cold exposure periods.

All sheep were kept in individual metabolic crates in temperature controlled chambers under continuous lumination. A ration of 1650g of brome-alfalfa pellets was offered daily.

The establishment of physiological cold stress was confirmed by a demonstrated 70% reduction in respiration rates and a 20-30% increase in oxygen consumption in both the AC and CC sheep. A decrease in body surface temperature and a 17% increase in heart rate were also measured in the chronic cold exposed sheep.

Microspheres of 15μm diameter and containing either Ce-141 or Sn-113 were used to obtain duplicate measurements of cardiac output and blood flow distribution in each sheep.

Technical difficulties prevented usage of three of the 24 sets of microsphere measurements and a further eight sets of data were partially incomplete due to displacement of injection catheters.

Estimates of cardiac output for the CON, AC, and CC exposed sheep were respectively 5.5, 6.5, and 6.1 liters per minute. These differences in cardiac output were not significant at the 0.05 level.

Relative to the control sheep, acute cold exposure caused an increase in blood flow (ml/100g tissue/min)to the thyroids, adrenals, and skeletal. muscle, while a decrease in blood flow was measured to the nasal turbinals, diaphragm, lungs (via bronchial arteries), radius and ulna bone tissue, and the gastrointestinal tract. Chronic cold exposure caused an increase in blood flow (ml/100g tissue/min) to the thyroids, nasal turbinals, and skeletal muscles, while a decrease was measured to the radius and ulna bone tissues, lungs (via bronchial arteries) and gastrointestinal tract.

These cold induced differences in blood flow are reflective of adaptive changes involving nutrient and heat distribution in the animals. Increased heat production via shivering or nonshivering thermogenesis in the skeletal muscles of the cold exposed sheep was possibly mediated by increased thyroid and adrenal activity. Evidence of possible increased heat conservation can be seen by the reduced blood flow to the extremities in the cold exposed sheep as well as the reduction in bronchial arterial flow.

The redistribution of gastrointestinal blood flow in the cold exposed sheep was primarily manifested as a reduced blood flow to the reticulo-rumen, and could potentially be a factor influencing digestive function and the absorption of nutrients in these animals.

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I. CHAPTER 1 :INTRODUCTION

An animal's thermal environment has been shown to influence in its efficiency of nutrient utilization and productivity (Kleiber, 1975). Many cold temperature induced changes in thermoregulation and thermoadaptive mechanisms in animals have been documented (Astrand and Rodahl, 1970; Bligh and Moore, 1972; Henshaw, 1978). Observable changes in digestive efficiency, primarily and practically demonstrated as a reduced feed digestibility, in cold exposed animals have also been reported (Warren etal. 1974; Westra and Christopherson, 1976). Ultimately, the energy budget of the animal is affected by these cold induced events which in turn affects the growth and production of the animal (Young, 1975). These factors become important to livestock producers because they affect the economic advantage of animal production in different geographical or climatic areas of the world.

In domestic ruminants, factors such as reticulo-rumen motility, rate of passage of digesta, and the influence or mediation of various endocrine hormones including thyroxin (T4), triiodothyronine (T3), adrenalin, and noradrenalin have been implicated in cold induced changes in animal production (Westra and Christopherson, 1976; Kennedy etal. 1977; Thompson etal. 1977). Also, the involvement of blood flow as it affects nutrient and heat transport and exchange is indicative of the significance of the circulatory system in thermal adaptation (Webster, 1974; Hales 1976a, b).

However, due largely to technical limitations, information concerning the role of blood flow has been difficult to obtain. Recent development of the radioactive microsphere technique has allowed some progress in measurement of blood flow in animals (Heymann etal. 1977). A few studies have been carried out on domestic ruminants under thermoneutral and acute cold conditions (Alexander, 1973; Hales, 1976a). However, to date, no studies have been undertaken to investigate the involvement of blood flow under long term or chronic cold acclimation conditions, in spite of the fact that major physiological changes are precipitated during cold acclimation.

The purpose of the present study was to use the radioactive microsphere technique to investigate blood flow changes occurring in domestic sheep as a result of acute and chronic exposure to cold. The results of such an investigation should aid in determining if differences in blood flow and blood flow distribution could account for digestive and thermoregulatory changes reported by other researchers . Understanding the nutrient and thermal transportation and exchange mechanisms could provide greater insight into the functioning of animals and consequently be of practical significance in designing or recommending animal feeding and management practices. This understanding may contribute to improving production in climatic areas where cold stress is a factor.

The findings in this thesis are presented in the text basically as two papers in a scientific journal form, chapters 4 and 5, with an accompanying review of literature, chapter 2. Appendix I and II contain supplementary methods and results not considered appropriate for inclusion in chapters 4 and 5. A general discussion and the conclusions are presented in chapters 6 and 7 of the thesis. Individual measurements for each animal are contained in appendix III.

A. CHAPTER 2 : REVIEW OF LITERATURE

2. 1 General Effects of Cold Exposure

Exposure of mammals to cold environmental temperatures has been shown to elicit many physiological changes which are discussed in comprehensive reviews by Hardy (1961), Webster (1974), Himms-Hagen (1978) and others. In general, physiological responses to cold include:

(1) peripheral vasoconstriction of blood vessels (Astrand and Rodahl, 1970; Anderson, 1977; Henshaw, 1977).

(2) an increase in heart rate and cardiac output (Thompson etal. 1975; Hales, 1976a).

(3) a decreased respiration rate and lower critical temperature (Webster etal. 1969a, b; 1974).

(4) an increase in the metabolic activity of the thyroid gland and adrenal cortex accompanied with increased circulating levels of thyroid and adrenal hormones (Guyton, 1969; Westra and Christopherson, 1976; Kennedy etal. 1977; Thompson etal. 1978).

(5) an increase in metabolic rate and thus heat production (Webster etal. 1969a, b; Astrand and Rodahl, 1970; Thompson etal. 1975; Depocas and Behrens, 1978).

The primary purpose or function of the above physiological changes is to facilitate an optimum thermoregulatory response to cold stress in order to maintain homeothermy. This is basically achieved by reducing heat loss and/or increasing heat production.

The mechanisms involved in cold induced increased heat production are basically two-fold, namely, (1) shivering thermogenesis and (2) nonshivering thermogenesis . An increase in heat production via shivering thermogenesis occurs primarily as a result of increased physical activity of skeletal muscle. Thermogenic effects of thyroid hormones and catecholamines have been reported in some animal species (Guyton, 1969; Depocas and Behrans, 1978; Foster and Frydman, 1978) however, the exact mechanism of nonshivering thermogenesis is yet unresolved.

A reduction in heat loss is primarily a result of reduced peripheral blood flow. Convective and conductive heat loss from the periphery is decreased as a consequence of functionally increasing the effective tissue insulation of an animal.

Although the above thermoregulatory mechanisms apparently vary in importance with different species, they are observed to be mostly universal in mammals. The control or integration of these responses is generally considered to occur via the hypothalamus (Webster, 1974), but again, the specific mechanisms of each remain largely speculative.

2. 2 Effects of Cold Exposure on Blood Flow

One major role of blood flow during cold exposure is the redistribution of heat within the body. As discussed by Federov and Shur (1942), Webster (1973; 1975), Hales

(1976a), Levey etal. (1977) and Henshaw (1978) , the redistribution of blood functions primarily as a heat conservation mechanism. The suggested processes include counter current exchange or, arterial-venous heat exchange (Astrand and Rodahl, 1970) and perhaps more important, vasoactive actions (vasoconstriction, vasodilation), involving arterial-venous blood shunting.

Edwards (1967), Webster (1974), and Hales (1976a) have reported that the vasoconstriction of the skin and extremities is a logical response to reduce conductive and convective heat loss by effectively increasing the tissue insulation of the animal. The extremities compose a large portion of the body surface (approximately 30%) (Webster, 1974; Bal, 1977) and , as such, can account for a large portion of the animal's heat exchange with the environment. Consequently, reducing the heat loss from the extremities is of considerable advantage to animals in a cold stress situation. However, to avoid cold damage, such as frost bite of tissues, a mammal must maintain at least a minimal blood supply to all tissues, including the skin and extremities. This blood supply must be sufficient to meet minimum nutrient and thermal demands to prevent freezing. Consequently, considering the relative lack of metabolically active tissue contained in the skin and extremities (Webster, 1974), and their high rate of heat loss, maintenance of temperature regulation in these tissues must be achieved largely via regulation of the blood supply.

During severe cold exposure, the blood supply regulation to surface tissues is observed via "hunting" reactions, or cyclic vasoconstriction-vasodilation of the extremities (Meyer and Webster, 1971; Levey etal. 1977; Henshaw, 1978).

Cold exposure also affects the blood flow to areas other than the skin and extremities. However, comprehensive information concerning the effect of cold exposure on blood flow in domestic ruminants is somewhat limited. An increase in blood flow to shivering skeletal muscles in acute cold exposed lambs and adult sheep has been reported by Alexander (1973) and Hales (1976a). The increased blood flow to these tissues was assumed to be due to the increased nutrient demand for shivering.

Alexander (1973) and Hales (1976a) also reported an increase in blood flow to the adipose tissue, which in the case of lambs, was interpreted as a direct thermogenic response of the brown fat tissue. In the adult sheep, the increase in blood flow to the adipose tissue is seen as part of a mechanism to mobilize fatty acids into the circulation for metabolic and thermogenic use (Thompson etal. 1975; 1978).

Although a significant increase in blood flow to tissues such as adrenals and thyroids of adult cold exposed sheep, signifying increased metabolic activity in adrenal and thyroid tissue, has not been shown, increased circulating catecholamine levels and T3, T4 levels have been demonstrated. (Webster etal. 1969; Westra and

Christopherson, 1976; Kennedy etal. 1977; Thompson etal. 1977). According to a review by Webster (1974), one of the effects of these elevated endocrine hormone levels is, by affecting cell membrane function, to enhance the oxidation of volatile fatty acids (VFA) in metabolically active tissues. Also the involvement of the liver in converting VFA, specifically propionate, to glucose for thermogenic purposes has been demonstrated (Thompson etal. 1975; 1978). The importance of portal blood flow concurrently affecting the distribution of VFA to the liver and the consequent distribution of glucose to metabolically active tissue has also been shown (Thompson etal. 1975; 1978)

Consequently, cold exposure can be seen to influence blood flow in animals via two basic processes; (1) By initiating the transport and redistribution of heat, particularly involving vasoactive and counter current mechanisms of the superficial tissues and (2) by influencing the mobilization and availability of nutrients, such as VFA, for conversion and oxidation in thermogenic tissues.

2. 3 Effects of Cold on Digestive Function in Ruminants

The reduction of digestibility of dry matter (DM), energy (E), acid detergent fiber (ADF), and nitrogen (N) feed components in domestic ruminants as a result of cold exposure has been repeatedly demonstrated (Blaxter and Wainman, 1961; Warren etal. 1974; Westra and Christopherson, 1976; Kennedy and Milligan, 1978). In combination with the need to maintain body temperature, the reduced digestibility of feed can be one of the major energy costs to the animal in a cold stress situation (Young, 1975).

Reduced digestibility as a result of cold exposure has been shown to be accompanied by several factors. An increased intake of feed necessitated by an increased energy demand has been reported (Baile and Forbs, 1974). An increased reticulo-rumen motility accompanied with an increased rate of passage of digesta, possibly mediated by thyroid hormones, has been demonstrated in cold exposed sheep (Westra and Christopherson, 1976; Kennedy etal. 1977). The concomitant effect of these physiological changes has been to reduce reticulo-rumen digesta volume in the cold exposed animals and to allow the passage of a more nutrient complete digesta to the intestinal regions of the gastrointestinal tract (GIT). One consequence of which , as observed by Kennedy and Milligan (1978), was to increase the apparent digestion of organic matter and non-ammonia nitrogen in the intestinal regions, which in part compensated for the reduced digestion in the reticulo-rumen.

The altering of digesta breakdown and absorption time at the various GIT sites influences the digestive function at those sites (Maynard and Loosli, 1969). The effect of cold exposure on digestive function can hence be viewed as causing an apparent reduction and redistribution of digesta absorption within the GIT.

2. 4 The Effects of Blood Flow on Digestive Function

Digestibility of feed or the amount of nutrients obtained from feed by a ruminant can be affected by many factors (Maynard and Loosli, 1969) . As was previously mentioned, the breakdown of digesta and the absorption of nutrients into the blood are two such factors.

The specific involvement of blood flow in the ruminant stomachs is primarily related to its effect on the transcapillary concentration or osmotic gradient. Hence the absorption and transport of hormones, substrates and nutrients to and from the tissues of the GIT are affected (Renkin, 1968; Engelhardt, 1970; Dobson etal. 1975; 1976).

From the foregoing discussion an interrelationship can be seen to emerge whereby digestive function, measured by feed digestibility, is affected by both cold exposure, and possibly blood flow. Also, blood flow is reportedly affected by cold exposure. Consequently, digestive functional changes observed in domestic ruminants as a result of cold exposure could be influenced by cold induced blood flow redistribution in and to the GIT.

Some studies have been made on regional blood flow in the GIT of ruminant animals at specific sites and under various experimental conditions (Sellers etal. 1964; Bell, 1967; Garbulinski, 1973; Webster, 1972; Symonds and Baird, 1973; Pamareto, 1974) . However, there has been no previous information available regarding the capillary blood flow to the entire GIT and fractional distribution therein. This lack of information is particularly relevant to further understanding of the effects of cold exposure on domestic ruminants. The lack of suitable research techniques has inhibited the study of capillary blood flow in animals. The problem in researching capillary blood flow to the GIT is consequently one of being able to utilize the appropriate investigative tool.

2. 5 Technical Methods of Investigating Blood Flow

As discussed in the previous sections, blood flow to specific areas of the GIT has been studied by several researchers. The more common techniques used in these studies have been: (1) indicator dilution methods involving both radioactive and nonradioactive markers (Symonds and Baird, 1973; Panareto, 1973; Setchell and Linzell, 1974) ; (2) thermal dilution method (Fegler and Hill, 1957; Webster and White, 1972) and (3) electromagnetic flow meters (Sellers, etal. 1964).

With the indicator dilution methods, catheters are placed "upstream" and "downstream" of a particular organ or tissue. A known amount of indicator is then injected or infused at a constant rate "upstream". Hence, by measuring the arterio-venous difference in indicator concentration, and uptake of indicator by the tissue, blood flow estimations can be obtained by calculations based on the subsequent "dye dilution curve". These calculations are based on the Fick principle. (Hamilin and Smith, 1977).

The thermal dilution method (Fegler and Hill, 1957; Webster and White, 1972) is basically identical to the indicator dilution technique with the unique exception that the indicator (a thermal difference), is often administered in the form of room temperature saline. Detection of thermal differences are consequently made via the use of a thermal sensing system of thermocouples or thermistors.

Both the indicator dilution and the thermodilution methods have the advantage of being relatively simple, at least in theory, to utilize and with the added option of repeatable measurements on an individual animal. However, catheter placement and patency are chronic problems with these techniques, and phlebitis is often encountered (Webster and White, 1972). Also, especially with the indicator dilution method, the reliability of results has been questionable due to selective uptake of indicator by tissues and apparent differences in measurements obtained by using different indicators (Foster and Frydman, 1978). Of course one of the limitations of these methods is that only one tissue or organ can be measured at one time.

The methodology for measuring blood flow via various electronic sensing systems is somewhat dependent upon the type of flow meter used. In principal these methods (Hamilin and Smith, 1977) involve the placement of a collar or probe around a blood vessel and alterations in the electromagnetic current, which are proportional to blood flow through the probe, can be recorded. The advantages of these techniques are th[illegible] instantaneous and accurate measurements can be obtained, repeated measurements can be made on one animal, and an estimation of pulse flow is possible. A few disadvantages are that surgical manipulation of the animal is required, restrictions of the natural vessel diameter are necessitated, the apparatus is somewhat bulky, and usually only one vessel can be monitored at one time.

The recent improvement of radioactive microspheres for use in microcirculation studies has provided a useful method for capillary blood flow research. Basically, this technique involves the injection of millions of radioactive plastic spheres into an arterial system one desires to measure. The microspheres are commonly injected into the left ventricle via a catheter inserted down the carotid artery, or directly via a hypodermic needle. With left ventrical injections, the fractional distribution of capillary blood flow to all tissues of the body can be estimated. The size of microspheres can vary between 3μm up to 50μm, however, the size most commonly used to measure capillary blood flow is 15μm±3.0 (mean S.D.) These spheres, behaving similar to oversized red blood cells, then are distributed via the arterial flow to the capillary beds of the body where they lodge. Consequently, by comparing the radioactive counts in a given amount of dissected tissue or organ, to the initial injected dose, an estimate of proportional blood flow to that tissue can be obtained. This technique has the clear advantage of being able to compare all tissues of the body simultaneously. Repeatable measurements are also possible by making repeated injections of microspheres containing different nuclide labels. Presently, there are commercially available at least eight nuclide labels which can be used in conjunction with microspheres. However, nuclide counting procedures become complex with more than two or three nuclide injections.

Disadvantages with the microsphere technique arise with the need to inject or infuse the spheres into the arterial system. This often requires surgical manipulation of the animal (Hales, 1973b). The need for nuclide counting equipment and the sacrificing of the experimental animals, to obtain tissues for counting, is also a usual disadvantage of the microsphere method. However, animal sacrifice is not essential for all microsphere research. For example, in the study of pulmonary circulation in humans low energy, short half life, serum and albumin microspheres are utilized (Buckberg etal. 1969; Heymann etal. 1977).

Comprehensive reviews and comparison of radioactive microsphere techniques have been provided by several reviewers (Buckberg etal. 1971; Hales etal. 1973b; Faichney and Hales , 1974; Heymann etal. 1977; Foster etal. 1978).

The investigation of capillary blood flows in ruminants utilizing the radioactive microsphere technique have been achieved by several researchers (Alexander etal. 1972; 1973; Hales, 1973b; Engelhardt and Hales, 1977). However, of these, only Alexander and Hales have obtained measurements of blood flow in cold exposed ruminants. Alexander's studies were on very young lambs while Hale's work involved comparisons on adult sheep exposed to warm and acute cold stress. Measurements of blood flow from the studies of Alexander and Hales are presented in detail in the following chapters where direct comparisons can be made with data obtained during the present study.

B. CHAPTER 3 :DESIGN OF THE STUDY

The purpose of the study was to measure various physiological parameters, including capillary blood flow, in wether sheep that had been exposed to warm, near thermoneutral conditions, and to acute and chronic cold conditions. A prerequesite for any experiment of this nature includes the standardizing of animals, and the development of a "plan of action" in which a premeditated chronological sequence of events is prescribed and followed. The following is a brief description of the series of events undertaken.

3. 1 Preparation Period

The experimental sheep were from a group of six -month-old wethers raised at the University of Alberta farm. During the preparation period of three weeks the animals were housed in free stalls with free access to a growing ration, water, and an iodized NaCl block. The growing ration consisted of approximately 50% rolled oats, and 50% brome-alfalfa pellets offered ad libitum. Their treatment during this period consisted of shearing (and every two weeks thereafter), treatment for external parasites by a sprayed application of "Lindane" (Benzene hexachloride 0.5%), recording of initial body weights, and the surgical preparation of exteriorized carotid loops (Hecker, 1974).

3. 2 Pre-Treatment Period

During this four week period the animals were kept initially for 7-10 days in individual metabolic crates while recovering from surgery. They received penecillin (5cc/day IM for 5 days) and were treated for internal parasites (2g Thiabendazole/45 kg body weight as a drench). An intramuscular injection of retinol, cholecalciferol, and alpha-tocopherol was also given. The surgical sutures used for the carotid loops were removed and the animals then transferred again to free stalls as in the preparation period. The sheep were, over this period of 28 days, introduced to a brome-alfalfa pelleted ration ad libitum and maintained on this diet for the duration of the experiment. One week prior to allocation to thermal treatments the animals were again returned to the individual metabolic crates in controlled temperature rooms (temp 18.7 C db, 11.6 C wb)

3. 3. 1 Thermal Treatment Period

During this phase of the experiment the animals were randomly assigned to one of three treatment groups each of which contained four animals. The thermal treatments consisted of control (CON), acute cold (AC), and chronic cold (CC), the relative temperatures and times of which are given in the methods section of chapter 4. All animals were offered 1625 g (air dry) of brome-alfalfa pellets per day, given in two equal servings at 0800 and 1700 hr daily.

Daily measurements of feed and water intake as well as weekly measurements of body weight and respiratory gaseous exchange were carried out on the CON and CC animals during the 10-12 week adaptation period. The measurement of apparent feed digestibility, via a total feces collection trial, and the measurement of body surface temperatures were also made.

3. 3. 2 Physiological Measurements and Microsphere Injections

Due to the time and equipment logistics, it was not possible to conduct the blood flow measurements on all animals at the same time. The measurements described in this section were consequently done on sheep one at a time, or, on occasion, in pairs.

During this phase, each animal in the three treatment groups, CON, AC, and CC, had been exposed to their respective thermal treatments for the prescribed period. The sequence of events, injection of microspheres and the measurement of associated physiological parameters are illustrated schematicaly in Figure 1.

Figure 1: Sequence of animal manipulations and measurements associated with microsphere injections and measurement of blood flow distribution in sheep exposed to near thermoneutral, acute cold and chronic cold temperatures

				PRE-INJ	INJ 1	BETWEEN INJ	INJ 2	POST INJ	
TREATMENT OR MANIPULATION		Catheter insertion	Feed removal (0900) Heart lead attach. Flush catheters	Cath. plac. Tamb. Halter Quiet period				Blood sample Resp. gas analy.	Terminate Dissection
MEASUREMENT		Body wt. Body temp		M1	M2	M1	M2	M3	Tissue counting
	AM	PM	AM	PM					
	PREVIOUS DAY		INJECTION DAY						SUBSEQUENT DAY

M1= measurement of blood pressure, heart rate, and respiratory rate
M2= measurement of cardiac output, heart rate, respiratory rate, and microsphere distribution
M3= measurement of hematocrit, hemoglobin, blood pressure, heart rate, respiratory rate, blood gas, body surface temperature, and respiratory gas analysis
Cath. plac.= catheter placement into left ventricle and right atria
Tamb.= attachment of tambour recording apparatus for the measurement of respiration rate
Halter= haltering the animal to the front of the metabolic crate

On the day prior to the microsphere injections, each animal was prepared with injection and sampling catheters. The catheters were inserted approximately 15-20 cm into the right jugular vein and left carotid artery, and thus at this stage were not in the chambers of the heart. Preparing the catheters one day previous to the microsphere injections reduced the need for handling the animals on the day of injections.

During the morning of the day of microsphere injections the animals were further prepared with electrocardiogram (EKG) recording leads, respiration rate measurement equipment, and the injection and sampling catheters were flushed with 10 ml of heparinized physiological saline.

During the pre-injection period (PRE-INJ) final catheter placements into the left ventricle and right atria were carried out. Physiological measurements (M1) of blood pressure, heart rate, and respiratory rate were made at this time and the animal was haltered in a fixed position to facilitate a consistent alignment of the head, neck and trunk. The animal was then left undisturbed for 20-30 min before the first microsphere injection was made.

The injection of Ce-141 and Sn-113 microspheres (INJ 1,INJ 2) and the measurement of cardiac output, heart rate, and respiration rate were then completed. Between the injection of the nuclides (BETWEEN INJ) the heart rates respiratory rates, and blood pressures were again measured. This between injection checking was used to indicate any changes in the "steady state" condition of the animal which may have affected the consistency of the results.

Following the injection of both radioactive microspheres, (POST INJ), final measurements (M3) of heart rate, blood pressure, and respiratory rates were taken as well as blood and respiratory gas samples for the determination of blood gas, hematocrit (Hct), hemoglobin (Hb), and oxygen consumption. Body surface temperatures were again measured at this time.

Sacrificing the animal, tissue dissection and nuclide counting was then carried out as described in the methods sections of chapters 4, 5, and appendix I. Specific individual animal results with regards to physiological parameters and blood sampling volumes are included in detail in appendix III.

C. CHAPTER 4 : THE EFFECTS OF ACUTE AND CHRONIC COLD EXPOSURE ON BLOOD FLOW DISTRIBUTION IN SHORN SHEEP

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Abstract

The effects of acute and chronic cold exposure on the distribution of cardiac output and blood flow were measured in six month old sheep (35-50kg) using 15μm radioactive microspheres containing Ce-141 and Sn-113. Four control sheep (CON) were exposed to 18±4.5C dry bulb (db), 11±2.8C wet bulb (wb) for 10-12 weeks. Acute cold (AC) and chronic cold (CC) treatment groups of four animals each were exposed to temperatures of 3±1.4C db, 0±0.8C wb, for 12 hr and 10-12 weeks respectively prior to and during measurements.

Confirmation of physiological cold stress was demonstrated in the cold exposed sheep by decreased respiration rates and by a 32% and 21% increase in rate of oxygen consumption in the AC and CC groups respectively. Decreased body surface temperatures and a 17% increase in heart rate were also measured in the chronic cold exposed sheep.

Estimates of cardiac output for the CON, AC, and CC

exposed sheep were respectively 5.5, 6.5 and 6.1 liters per minute. Relative to the control sheep, acute cold exposure caused an increase in blood flow (ml/100g tissue/min) to the thyroids, adrenals, and skeletal muscle while a decrease was measured in blood flow to the nasal turbinals, diaphragm, lungs (bronchial arteries), radius and ulna bone tissues, and the gastrointestinal tract. Chronic cold exposure caused an increase in blood flow to the thyroids, nasal turbinals, and skeletal muscles, while a decrease was measured to the radius and ulna bone tissues, lungs (bronchial arteries) and gastrointestinal tract.

Introduction

Blood flow distribution is important in nutrient transportation and thermal distribution and exchange in mammals (Kleiber, 1975; Hales, 1976b). During cold exposure heat exchange mechanisms involving arterial-venous heat transfer or counter current exchange mechanisms (Astrand and Rodahl, 1970) and blood shunting by vasoactivity (vasodilation, vasoconstriction) have been reported to be functionally important in thermoregulation (Webster, 1973; 1975; Hales, 1976a; Levey etal. 1977). The resulting transfer of heat from the shivering muscles and viscera to the skin and extremities is thermally advantageous to the animal in maintaining its body temperature (Federov and Shur, 1942; Hales, 1976a). Primarily, these thermoregulatory actions function to optimize heat conservation while concurrently preventing cold tissue damage to the skin and extremities.

The development of metabolic changes as a consequence of chronic cold acclimation is well established in mammals (Webster, 1974). There is however, little understanding of the involvement and influence of circulatory mechanisms of blood flow during these metabolic changes. Suitable investigative methods for measuring capillary blood flow have been lacking. Recently the radioactive microsphere method, see reviews by Hales 1973b and Foster 1978, has been found to be valuable and reliable for investigating capillary blood flow and consequently has received increasing use in studies on domestic ruminants by several researchers (Alexander etal. 1972; 1973; Hales, 1973b; Engelhardt and Hales, 1977). However, only a few investigations have been made on animals exposed to thermal stress (Alexander, 1973; Hales, 1973a; 1976a, b) and to date, no studies have investigated blood flow changes occurring as a result of long term or chronic acclimation to cold. The purpose of the present study was to use the radioactive microsphere technique to investigate blood flow and blood flow distribution in domestic sheep and changes occurring as a result of acute and chronic cold exposure.

Methods

Animals, Preparetion and Treatment

Twelve crossbred wether sheep, six months of age and weighing 35 to 50 kg were used. At the start of the study, all sheep were treated for internal and external parasites and received injections of retinol, cholecalciferol, and alpha-tocopherol. To ensure a relatively constant fleece depth, the sheep were shorn every two weeks to a fleece depth of approximately 1 cm. Six to eight weeks prior to the allocation to treatment groups, carotid skin loops were established on the left common carotid artery of each sheep according to the surgical methods described by Hecker (1974).

All animals were housed in individual metabolic crates in climatic controlled rooms under constant lighting conditions, prior to and during the thermal treatments and blood flow measurements.

A ration of 1650g (air dry) of brome-alfalfa pellets (Bromus inermus, Medicago sativa) was offered in equal 825g servings at 0800 and 1700 hr daily (dry matter 91.65%, acid detergent fiber 49.99%, energy 4.493 Kcal/g, crude protein 14.23%). The ration was ground through a 5mm screen before pelleting to 3mm diameter. The sheep had free access to water and iodized block salt.

The sheep were randomly assigned to three groups of four animals each. The temperature treatments were as follows: (1) Control animals (CON) were conditioned for 10 to 12 weeks to 18.7 (range 13.3 to 22.2) C dry bulb (db) and 11.6 (range 10.5 to 16.1) C wet bulb (wb); (2) The acute cold group (AC) was initially conditioned to 18 C db, 11 C wb for six weeks followed by 12 hr exposure to 3 (range 1.6 to 4.4) C db and 0 (range -1.6 to 2.2) C wb; (3) The chronic cold animals (CC) were exposed for 10 to 12 weeks at 3.3 (range 1.6 to 4.4) C db and 0 (range -1.6 to 2.2) C wb.

Catheterization

To facilitate the injection of microspheres, a Tygon catheter (Tygon microtubing, 0.04-0.07mm ID, Norton Plastics, Akron Ohio, U.S.A.) was inserted into the carotid artery of each sheep 24 hr prior to the microsphere injections. The previously established carotid loops allowed catheter insertion under local anesthetic thus avoiding the need for the more radical surgical manipulation used in previous investigations (Hales 1973a, b).

The constant withdrawal of arterial blood, during the microsphere injection phase, as per the artificial organ technique, for the calculation of cardiac output (CO) (Hales, 1973b) was accomplished by inserting a catheter approximately 10cm cephalically into the exteriorized left common carotid artery. In preliminary tests, the femoral artery as used by Hales (1973b) for the artificial organ blood withdrawal was found to be unsatisfactory for providing consistent withdrawal rates of greater than 15 ml/min considered necessary for the present study.

Venous blood sampling for estimating blood flow via arterial venous anastomosis (AVA) was obtained by placing a Tygon catheter (0.04-0.07mmID) into the right atria via the right jugular vein. A mixed venous blood sample was consequently obtained for AVA calculations as described by Hales (1973b).

Catheter placements were established with the aid of a Sanborn pressure transducer (Model 267BC) to identify characteristic blood pressure waves of the respective blood vessels and heart chambers.

Microsphere Injections

Gamma emitting Ce-141 and Sn-113 radioactive microspheres were used in the study (15μm±3, New England Nuclear, Boston, Massachusetts). Each nuclide injected seperately contained approximately 4 X 10^5 microspheres per kg body weight. Later statistical analysis demonstrated no significant difference between tissue distribution of radionuclide type. Each injection of microspheres had approximately 0.22 mci of activity and were contained in 10 ml of 10% dextran with a trace of Tween 80 suspending agent.

Plastic syringes (20ml) were used to inject the spheres over timed intervals of 60 to 90 seconds. Withdrawal of blood for CO and AVA estimates were also into plastic syringes (50cc) and taken over timed intervals of 120 to 150 seconds starting simultaneously with the start of microsphere injections. A Harvard constant infusion/withdrawal pump (Harvard Apparatus, 150 Dover Rd. Mills, M. A.) was used and calculations of CO and AVA was according to the methods of Hales (1973b).

Tissue Counting

Within 24 hr of injection of the microspheres the animals were terminated by an overdose of sodium pentabarbital I. V.. Selected organs and tissue samples were dissected out, weighed and stored at -10 to -20 C before counting for radioactivity.

The gamma counting system used in this study consisted of two 7.5 by 7.5 cm NaI crystals with an Ortec #456 high voltage supply (Ortec Inc. Oak Ridge Tennessee, U. S. A.), Canberra amplifier#1417B, (Canberra Ind. Inc., Meriden, Connecticut, U. S. A.) and a Canberra scaler #1437. The counting system was calibrated using a known activity of Ce-141 and Sn-113 nuclide source. All blood sample and injection counts were appropriately adjusted for nuclide residue in the injection catheters and syringes.

Other Measurements

Heart rates were measured using a Sanborn physiological recorder model 7714-04A. The lead system was positioned on the right and left thorax and dorsal midline. Blood samples for hematocrit determination were taken from the left common carotid artery and analysed by a microhematocrit method.

Respiratory Measurements

All respiratory gas analysis was done using a Beckman respiratory gas analyser (Beckman Instruments) in conjunction with an open air current system utilizing a respiratory hood, as described by Young etal. (1974). Respiratory rates were measured using a thorax pneumograph and recording tambour.

Skin Temperature

Skin surface temperatures reported as trunk (average of back, flank, and midside) and extremities (average of ears and lower front leg), for the chronic cold and control animals were measured during five consecutive days immediately prior to the microsphere injections. These measurements were made using a thermocouple system (model BAT-8, Bailey Instruments Inc., Saddle Brook, U. S. A.).

Statistical Analysis

Significant differences between means for treatment groups were tested by analysis of variance and Student-Newman-Keuls multiple range test (Steel and Torrie, 1960). Where disproportionate subclass numbers occurred in a treatment comparison, the analysis of variance was conducted according to a least squares unequal numbers analysis.

Results

Both acute and chronic cold exposure of the sheep resulted in an increase in the rate of oxygen consumption of 32% and 21% for the AC and CC animals respectively (Table 1). A significant decrease ($P<0.05$) in respiration rate was also measured in the AC and CC animals. Heart rates were not significantly different between the CON and AC sheep, however, a significant increase in heart rate of 17% was observed in the CC treatment group. A 15% and 10% increase in cardiac output was measured in the AC and CC sheep respectively, although these increases were not stastisticaly different from the control animals at the 0.05 probability level. Increases in hematocrit of 22% and 2% for the AC and CC groups respectively also occurred with the increase in hematocrit in the AC sheep being significantly greater at the 0.10 probability level. Body surface temperatures for the trunk and extremities were significantly reduced in the CC animals.

Table 1: Cardiorespiratory functions, skin temperature and body weight of sheep exposed to near thermoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures

	CON	AC	CC	SEM
Body weight (kg)	40.5	44.8	39.8	2.6
Heart rate/min.	131^{e}	120^{e}	157^{d}	12.2
Respiratory rate/min.	73^{a}	22^{b}	22^{b}	7.0
Cardiac output (liters/min.)	5.5	6.5	6.1	0.79
Oxygen consumption (ml/min.)	316.2^{b}	465.7^{a}	397.9^{a}	35.5
Hematocrit %	25.3^{e}	32.3^{d}	25.8^{e}	2.2
Mean Body Surface Temperatures (C)				
Trunk	34.6^{a}		31.2^{b}	1.23 *
Extremities	33.1^{a}		20.6^{b}	2.11 *

Values are based on means from four sheep per treatment group except for the cardiac output values which are from two, four, and two sheep for the CON, AC, and CC treatments respectively. Each sheep mean was derived from one to three observations per animal.

$^{a, b, c}$ Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

$^{d, e, f}$ at $P<0.10$ probability levels.

* Statistically compared using Students paired T test.

SEM is the standard error of treatment means.

For the injection of microspheres, between 30 to 40 cm of catheter tubing was inserted via the carotid artery to position the tip of the catheter in the left ventricle. Furthermore a consistent orientation of the head, neck, and trunk was neccesary, requiring each animal to be haltered in a fixed position during catheter insertion and microsphere injections. Catheter positions were verified during the subsequent dissection of the animal and by examination of the relative microsphere radioactive counts in selected regions of the body. By comparing radioactive counts in the heart muscle, brain, kidney and gastrointestinal tract for example, zero or abnormally low counts in the heart and brain was clearly indicative of an incorrect positioning of the injection catheter at the time of microsphere injections. In eight of the 24 injections, low numbers of counts were found in heart muscle and the brain. On these occasions the catheter was found, upon post mortem examination, to be positioned in the descending aorta. The relative distribution of microspheres to all organs and tissues supplied via the descending aorta were nevertheless found to be consistent with data obtained from sheep with left ventricle placed catheters. However, no blood flow or distribution values could be obtained for tissues from the anterior or forequarter areas of these animals.

Blood Flow and Blood Flow Distribution

Table 2 illustrates cardiac output distribution to whole organs among the three thermal treatment groups. A comparison of blood flow (ml/100g tissue/min) among the three thermal treatment groups used in the present study as well as values reported by other researchers for acute cold and warm or thermal neutrally exposed domestic sheep are presented in Table 3. An index of preferential blood flow distribution and treatment differences is presented in Table 4, and is referred to as "relative blood flow". Relative blood flow is a comparison of blood flow to a tissue in relation to the overall body average blood flow to an equal weight of tissue (cardiac output/body weight). Hence, a tissue receiving an average amount of blood would have the value 1.00, whereas a tissue with preferential or greater than average blood flow would have a value greater than 1.00.

Discussion

Evidence of physiological cold stress was seen in the cold exposed sheep by observed decreased respiration rates and surface temperatures and a 20-30% increase in oxygen consumption. A 22% increase in hematocrit and a 17% increase in heart rate were also measured in the AC and CC sheep respectively.

Table 3 summarizes blood flow estimates to selected organs and tissues in control (thermoneutral) and cold exposed sheep. Both previously published results found in the literature as well as results for the present study are shown. In the control animals used in the present study lower blood flow values are seen specifically to the spinal cord, thyroids, spleen, skeletal muscle, skin and nasal turbinals. These differences may be owing to the differences in sampling sites used in the various studies. For example, specific anatomical sites from which the spinal cord, muscle, and skin samples were taken in the various studies is not always reported. Also the coefficient of variation reported in the literature values range from 5% to 30% in these tissues and may in itself be accountable for the apparent discrepancies.

The thyroids of all the animals in the present experiment demonstrated a much reduced blood flow compared to published values. This perhaps relates to the method of microsphere injection. A common procedure in microsphere experiments of this nature is to place the injection catheter in the left ventricle via direct surgical catheterization of the carotid artery, within 24 hr of microsphere injection. Consequently, at least a minimal degree of inflammation at the site of surgical incision would be expected. Hence, as is discussed by Smith and Hamilin (1977), an excessive increase in blood flow due to the inflammation of the ventral neck area of the animals, including the thyroid arteries, would likely result. An increase in thyroid blood flow would consequently occur. This hypothesis might further be supported by the observed higher spleen blood flow in the literature values, possibly indicating an increase in blood phagocytosis occurring in this organ. In the present experiment, use of the previously established carotid loops may have reduced the inflammatory effect thereby reflected in the thyroid and spleen blood flow measurements. However, the coefficient of variation in the literature values for thyroid and spleen is between 10-25% and could again possibly account for large differences reported in the different studies.

With regard to the nasal turbinals, in the present experiment this tissue sample was inclusive of the bone and upper jaw of the animal, and consequently may account for the comparably lower flow values measured in this tissue. Other authors did not indicate the proportion of bone or epithelium tissue present in their samples.

The liver blood flow estimates deserve a special comment. Although the microsphere technique will give an estimate of arterial blood flow to this organ, this may not be a measurement of total hepatic blood flow. Any microspheres shunted via mesenteric AVA's can, and likely do, lodge in the liver. Consequently, unless the portal vein is catheterized and a representative sample of blood is withdrawn as per the calculation of AVA's in the right atria (Hales 1973b; 1974) the number of microspheres contributed to the liver via mesenteric AVA's is unknown. This may bias liver arterial flow measurements.

An additional point to be noted is that with the control animals in the present study (CON), technical difficulties with the blood withdrawal system resulted in cardiac output estimates in only two of the control animals. Consequently, care must be taken in interperating control (CON) sheep blood flow values in Table 4.

Blood Flow Redistribution

Skeletal Muscle

One aspect of cold induced redistribution of blood flow observed in the present study was an increased flow to the skeletal muscles of the acute and chronic cold exposed sheep. An increase in muscular blood flow has also been observed by Hales (1976a) in acute cold exposed sheep and it is suggested by Hales to be due to the energy demand of shivering muscle. Of particular interest is that the continued or maintained increase in nutrient blood flow to the skeletal muscle, observed in the present experiment with regard to the CC animals, may reflect the involvement of this tissue in nonshivering thermogenesis. Skeletal muscle has been reported to contribute to nonshivering thermogenesis in other species (Webster, 1975b; Jansky, 1976; Foster etal. 1978) and may be of significance to adult, chronic cold exposed sheep.

Internal Organs and Glands

In the present experiment increased blood flow to the thyroids and adrenals was the main blood flow alteration seen in the internal organs and glands caused by the cold exposure. The increase in blood flow to tissues such as thyroids and adrenals as well as skeletal muscle in the cold exposed animals suggests an increase in metabolic activity in these tissues. Although physiological control mechanisms involved in thermoregulation and metabolism will not be discussed in detail in this report, these tissues have been implicated in thermoregulation by several researchers (Alexander etal. 1972; Westra and Christopherson, 1976; Kennedy etal. 1977; Satinoff, 1978; Depocas and Behrans, 1978). The increased metabolic activity signified by increased capillary or nutrient blood flow seen in the present study, would support these suggestions. This occurrence would be logical considering that increases in circulating catecholamine and thyroid hormone levels have been reported in cold exposed sheep (Westra and Christopherson, 1976; Thompson etal. 1977; Christopherson etal., 1978) which would likely involve an increase in metabolic activity in these tissues.

Adipose, Skin and Bone Tissue

The apparent mobilization of fat sources signified by increased blood flow to the adipose areas observed by Alexander in 1973 and by Hales (1976a) was not as obvious in the present experiment. Nor was the reduction in skin blood flow, signifyfng vasoconstriction, as apparent in the present study despite the decreased surface temperatures.

Reduction of blood flow to the extremities of the AC and CC animals in the present study was only observed in the radius and ulna bone tissues, and the hooves. It is likely that part of the discrepancy in these results is due to the differences in temperature to which the animals were exposed. In previous studies. Alexander etal. (1973) exposed lambs to -10C compared to 3C db 0C wb in the present study. Consequently it is feasible that a "hunting" response was initiated in the lambs Alexander studied. However, such a response may not have occurred at 3C db, 0C wb in the present study. Hence one might expect that Alexander's lambs were measured at a vasoconstrictive phase of skin blood flow which may have caused a reduced blood flow measurement compared to his control or warm animals. Also, in the present study, both cutaneous and subcutaneous skin layers were measured together. Consequently, if there was a reduction in capillary blood flow to the cutaneous layer, the reduction would not have been measured.

The apparent increased mobilization of fat observed by Alexander (1973) may also be accounted for by the fact that due to the lower temperature and due to the smaller, younger animals, the lambs may have been considerably more cold stressed than the sheep in the present study.

However, the importance of skin blood flow in affecting the redistribution of blood in cold exposed animals is considerable. According to Bal (1977) in terms of bulk, the skin is the largest organ of the body. Consequently even a small change in blood flow to the skin would result in a significant effect on blood flow redistribution. This is clearly stated by Guyton (1969) where he reports that the rate of blood flow to the skin plexus in humans can be altered as much as 100 fold. This would represent a difference of between 20-50 ml/minute to the entire skin surface, up to 2-3 liters/minute, depending on the degree of vasoconstriction and vasodilation.

In the present experiment, the varying skin temperatures in the CC animals (Table 1) would be indicative of a certain degree of skin vasoconstriction occurring. This is likely a significant factor influencing blood flow redistribution in these chronic cold exposed sheep.

Respiratory System

The respiratory rate in both AC and CC exposed sheep was observed to decrease. However, oxygen consumption increased in these groups. Consequently, one can argue that although the respiration rate in these animals is reduced in frequency, that oxygen extraction and depth of respiration in increased.

It was also observed in the present experiment that both the AC and CC exposed sheep demonstrated a reduced blood flow (ml/100g/minute) to the lungs (bronchial capillary flow) although this would seem to be in conflict with enhancing oxygen absorption from the lungs, reduced bronchial blood flow would perhaps result in a reduced heat loss from the upper respiratory passage, which may be more of a priority to the animal. This may be especially relevant for the AC stressed sheep. Furthermore, microspheres measure only bronchial capillary flow, which is not a direct measurement of alveoli blood flow and oxygen exchange.

Gastrointestinal Tract (GIT)

As was observed by Hales (1976a) and is supported by our findings, blood flow (ml/100g/minute) to the entire or whole GIT (reticulo-rumen, omasum, abomasum, small intestine, and large intestine including colon) did not change appreciably in the AC stressed sheep. However, redistribution of GIT blood flow can be seen. In both the AC and CC exposed animals the present experiment demonstrated that the percent distribution of CO to the GIT was reduced. This seems to be owing primarily to the reduced percent distribution of CO to the reticulo-rumen in these animals. Also, this reduction to the reticulo-rumen is a real reduction in terms of blood flow (ml/100g/minute), as can be seen in Table 4. The implications of these GIT blood flow changes or redistributions are discussed in more detail in Chapter 5.

Arterial Venous Anastomosis (AVA)

In the present experiment, AVA blood flow was significantly reduced in the AC and CC sheep. This would seem logical, especially in the case of the CC sheep where a measured reduction in peripheral AVA blood flow signified by reduced surface temperatures was measured. However, the total amount of blood being shunted from the capillary circulation via AVA's in all treatment groups represented a relatively small percentage of CO (less than 1%) Consequently, in the present study, the AVA blood flow can be considered of lesser significance in the cold exposed sheep in influencing thermoregulation, or blood flow distribution. Conversely, as is pointed out by Hales (1973a, 1976b), AVA blood flow may be of considerable influence under different environmental conditions, such as heat exposure.

Acute Cold Exposure versus Chronic Cold Acclimation

The present study has shown certain differences or magnitude of changes in organ or tissue blood flow between the 12 hour, acute cold exposed and chronic cold acclimated animals. Compared to the CC animals, blood flow differences are illustrated in the AC animals by notably higher blood flow (ml/100g/minute) to the adrenals and abomasum and lower blood flow to the lungs (bronchial arteries), diaphragm, nasal turbinals, and skeletal muscle. These differences between AC and CC exposed animals may reflect the relative metabolic activity and functional importance of these tissues between the two cold exposures. As was previously mentioned, the involvement of skeletal muscle in nonshivering thermogenesis may be of increasing importance in the cold acclimated sheep. Also, especially in the case of reduced blood flow to the lungs (bronchial arteries), diaphragm, and nasal turbinals, these differences would seem to be indicative of heat conservation mechanisms in the AC animals.

Relative Blood Flow

A relative comparison of blood flow values (Table 4), illustrates the preferential blood flow supply to the various tissues as a result of the cold treatments. Although the relative blood flow values reflect the most metabolically active tissues, to be totally comprehensive of the cold treatment effects, the total tissue or organ weights must also be considered. For example, although the skeletal muscles demonstrate comparatively low relative blood flow values the fact that they comprise approximately 27% of the body weight (Hales, 1976b) may indicate that a small increase in blood flow per gram in this tissue is relatively significant.

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NOTE: References for this chapter are contained in the thesis bibliography.

Table 2: Percent distribution of cardiac output per organ in sheep exposed to near thermoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures

	CON	AC	CC	SEM
Internal Organs and Glands				
brain	1.08	1.39	1.69	0.111
heart	3.77^{d}	2.35^{e}	3.97^{d}	0.265
lungs (bronchial arteries)	6.20^{a}	0.65^{b}	1.19^{b}	0.255
liver	1.89	0.18	1.02	0.674
kidney	4.18	6.25	4.45	0.756
thyroids	0.06^{b}	0.13^{a}	0.10^{a}	0.015
pancreas	1.48	1.12	1.63	0.280
adrenals	0.20^{a}	0.07^{b}	0.15^{ab}	0.034
gall bladder	0.03	0.02	0.02	0.005
urine bladder	0.04		0.03	0.037 *
spleen	1.29^{b}	1.98^{a}	1.43^{b}	0.391
Gastrointestinal Tract				
esophagus	0.15	0.16	0.10	0.039
reticulorumen	6.95^{d}	3.36^{e}	4.21^{e}	0.536
omasum	1.63	1.17	1.06	0.174
abomasum	3.56	3.61	3.13	0.558
small intestine	8.55	7.71	6.70	0.867
large intestine	5.70	4.63	4.25	0.439
Other				
back hooves	0.012	0.007	0.005	0.002
front hooves	0.013	0.007	0.005	0.001
ears	0.02	0.02	0.11	0.032

$^{a,\ b,\ c}$ Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

$^{d,\ e,\ f}$ at $P<0.10$ probability levels.

* Statistically compared using Students paired T test.

SEM is the standard error of treatment means.

Table 3: Estimates of tissue and organ blood flow (ml/100g/min) in sheep exposed to near thermoneutral (Control), Acute Cold and Chronic Cold temperatures

	Control					Acute Cold		Chronic Cold	
	Hales 1973a	Hales 1973b	Hales 1976a	Engelhardt 1977	Present study	Hales 1976a	Present study	Present study	SEM
Species and Age	AS,1-3Y	AS,1-3Y	AS,2-3Y	AS,1-3Y	AS,6M	AS,1-3Y	AS,6M	AS,6M	
Body Weight	22-32kg	22-32kg	23-28kg	22-32kg	35-50kg	23-28kg	35-50kg	35-50kg	
Environment Temp. C	19C	19C	19C	18C	18C	5C	3C	3C	
Internal Organs and Glands									
brain	63± 4.5	70± 4.9	61± 7.2		63.9	61± 3.2	83.1	113.8	13.80
spinal cord	13± 1.1	16± 1.1	16± 0.7		4.6	16± 0.7	18.5	22.1	5.60
heart	161±47.1	154±24.5	108± 8		116.2	156±15.0	70.7	103.3	15.60
lungs (bronchial arteries)					57.5^{a}		5.9^{b}	12.2^{b}	3.93
liver					6.9		1.5	6.2	6.82
kidney		551±46.8	382±53		187.6	411±40	319.3	183.8	39.52
thyroids	195±45.6	462±97.9	302±71		48.8^{e}	265±42	97.6^{d}	88.1^{e}	24.46
pancreas					168.4		224.0	188.1	35.13
adrenals	158±34.8	219±22.1	204±26		140.5^{ae}	245±26	202.4^{ad}	108.2^{be}	31.50
gall bladder					4.8		8.3	7.1	1.72
urine bladder					12.3			5.9	4.83
spleen	198±22.5	234±33.2	147±35		68.7	123±28	115.1	79.9	25.56
Muscle									
longissimus dorsi	6± 2.2		2.3±.4		1.7	5.1±1.4	5.9	8.2	0.10
gluteus maximus	6± 2.2	5± .8			3.0^{c}		5.6^{b}	14.3^{a}	1.30
triceps					1.2^{f}		5.0^{e}	16.6^{d}	1.30
diaphragm		8.7±1			28.6^{a}	12.9± 1.5	11.3^{b}	28.2^{a}	2.60
Gastrointestinal Tract									
esophagus					14.1		22.4	15.5	0.55
reticulo-rumen	72±21.6	68±10.4	37± 5.7	73.3±21	56.1^{d}	33.5± 2.8	34.0^{e}	37.8^{e}	6.41
omasum			68±16	85.1±63	69.9	46±3.9	57.8	54.1	9.32
abomasum			117±26		88.1^{ed}	137±23	136.0^{d}	73.2^{e}	17.89
small intestine	128±13.5	130±13.8	99±20		71.2	101±17	83.3	50.1	10.35
large intestine	59±17	64±10.7	70±9.2		63.3	72±5.4	44.8	53.1	7.21
Skin									
midside	14± 1.3	18± 1.5	16± 3.7		5.6	12± 1.8	12.0	7.8	1.20
nasal					11.0		7.1	11.5	3.10
lower front leg	7± 2	5± .8	7.8± 2.5		1.1	0.5± .1	1.1	0.9	0.60
Fat									
subcutaneous					4.2		1.6	2.8	0.77
kidney fat		5.3± 2			10.2	31.3±6.4	5.9	5.1	1.75
Bone									
radius and ulna					1.3^{d}		0.2^{f}	0.5^{e}	0.05
nasal turbinals	22± 2.1	28± 5.2	24± 5.5		12.3^{e}	7.8± 1.4	4.2^{f}	19.7^{d}	5.30
Other									
back hooves					1.0		0.5	0.4	0.05
front hooves					0.8		0.5	0.4	0.05
ears	9± 1.5	6± 1			8.5^{a}		2.7^{b}	8.5^{e}	3.20

AS = adult sheep, M = month, Y = year

a, b, c Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

d, e, f at $P<0.10$ probability levels.

SEM is the standard error of treatment means in the present study.

Table 4: Relative blood flow in sheep exposed to near themoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures [ml blood flow per 100 g tissue weight divided by ml blood flow per 100 g body weight (average tissue = 1)]

	CON	AC	CC	SEM
Internal Organs and Glands				
brain	4.77	5.84	7.31	1.32
spinal cord	0.37	2.06	1.25	0.46
heart	8.61	4.41	6.76	1.10
lungs(bronch.art.)	2.57	0.41	0.87	0.78
liver	0.49	0.10	0.40	0.15
kidney	13.55	21.49	11.83	2.53
thyroids	3.78	7.15	5.82	2.02
pancreas	11.79	15.88	12.19	2.12
adrenals	9.83^{d}	14.10^{d}	7.08^{e}	2.25
gall bladder	0.84	0.53	0.46	0.11
urine bladder	0.92		0.39	0.69
spleen	5.16	7.30	5.14	1.68
Muscle				
longisimus dorsi	0.14	0.33	0.59	0.18
gluteus maximus	0.24^{b}	0.36^{b}	0.99^{a}	0.10
triceps	0.10^{c}	0.38^{bc}	1.19^{a}	0.27
diaphragm	3.01^{a}	0.78^{b}	1.12^{b}	0.41
Gastrointestinal Tract				
esophagus	1.07	1.40	1.02	0.30
reticulorumen	4.00^{a}	2.17^{b}	2.45^{b}	0.36
omasum	5.04	3.86	3.54	0.59
abomasum	6.36	8.84	4.72	1.06
small intestine	5.17^{d}	5.47^{d}	3.24^{e}	0.61
large intestine	4.53^{a}	2.88^{b}	3.48^{ab}	0.42
Skin				
midside	0.44	0.73	0.52	0.22
nasal	0.83	0.54	0.68	0.22
lower front leg	0.09	0.05	0.05	0.02
Fat				
subcutaneous	0.30	0.12	0.19	0.06
kidney	0.74	0.50	0.32	0.13
Bone				
radius and ulna	0.10^{a}	0.01^{b}	0.03^{b}	0.01
nasal turbinals	0.93	0.37	1.18	0.28
Other				
back hooves	0.08^{a}	0.03^{b}	0.03^{b}	0.01
front hooves	0.06^{a}	0.03^{b}	0.03^{b}	0.01
ears	0.18	0.17	0.57	0.23

$^{a, b, c}$ Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

$^{d, e, f}$ at $P<0.10$ probability levels.

SEM is the standard error of treatment means.

D. CHAPTER 5 :GASTROINTESTINAL BLOOD FLOW DISTRIBUTION IN COLD EXPOSED SHEEP

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Abstract

The influence of acute and chronic cold on the distributions of cardiac output to the gastrointestinal tract were measured in six-month old sheep (35-50 kg) using radioactive microspheres containing Ce-141 and Sn-113.

Groups of four sheep were exposed in controlled temperature chambers to either (1) Control temperature, 18C dry bulb (db), 11C wet bulb (wb) for 10-12 weeks ; (2) Acute cold, conditioning to 18C for six weeks followed by exposure to 3 Cdry bulb for 12 hr immediately prior to measurement ; (3) Chronic cold, 3C db, 0C wb, for 10-12 weeks.

In the control, acute cold and chronic cold treatments respectively 26.4%, 20.5% and 19.4% of cardiac output was distributed to the gastrointestinal tract. The percent of cardiac output reaching the reticulo-rumen, omasum, abomasum, small intestine, and large intestine (caecum plus colon) were respectively 6.5, 1.3, 4.3, 9.3, and 5.0 for the control group ; 3.4, 1.2, 3.6, 7.7, and 4.6 for the acute

cold exposed group; and 4.2, 1.1, 3.1, 6.7 and 4.3 for the chronic cold exposed group.

Estimates of cardiac output were respectively 5.5, 6.5, and 6.1 liters per minute for the control, acute cold and chronic cold exposed sheep. Consequently, considering the differences in cardiac output, the average total amount of blood flow (ml/100g tissue/min) to the total gastrointestinal tract was not significantly different among the three groups. However, there was a significant decrease in blood flow to the reticulo-rumen of the acute cold and chronic cold exposed sheep (34.0 and 37.8 ml/100g/min for the acute and chronic cold sheep versus 56.0 ml/100g/min for the control sheep). Conversely an increase was observed in blood flow to the abomasum of the acute cold exposed sheep, (136.0 ml/100g/min versus 88.1 ml/100g/min for the control sheep).

Introduction

Cold stress in domestic ruminants has been seen to cause several physiological changes. Among those changes, the alteration of nutrient metabolism and utilization of feed or, digestive functional changes have been suggested to be of particular significance to livestock production (Young, 1975). Westra and Christopherson (1976) suggested that the cold induced changes in ration digestibility may be due at least in part, to a measured increase in the rate of passage of digesta and increased reticulo-rumen motility.

This increased rate of passage of digesta and motility has been most recently demonstrated by Kennedy etal. (1976); (1977) and Kennedy and Milligan (1978) in cold exposed sheep. A reduction in retention time of digesta in the GIT could consequently reduce or change the nutrient absorption time at specific gastrointestinal sites, with the resulting reduction in feed digestibility.

The absorption of nutrients from the digesta or the movement of nutrients across epithelial linings at various sites of the GIT has been shown to be parallel to, or largely dependent upon blood flow to the absorption sites (Renkin, 1968; Dobson etal. 1976). There have been several attempts to study the circulation dynamics of metabolites absorbed from the GIT in ruminants (Conrad etal. 1958; Conner and Fries, 1960; Renkin, 1968; Katz and Bergman, 1969; Anderson etal. 1969; Levin, 1969; Webster, 1975; Dobson, 1976a, 1976b; Edrise, 1977). However, the measurement techniques employed by researchers, such as thermal or dye dilution, radioactive and non-radioactive markers, and flow meters for example, have been technically restrictive and thus limited progress.

Only more recently, the use of radioactive microspheres (Hales etal. 1976 and Engelhardt and Hales, 1977) has enabled a more complete measurement of capillary blood flow.

The present study was undertaken to investigate the effects of acute and chronic cold conditioning on the distribution of blood flow to the GIT in sheep. The influence of blood flow on digestive functional changes in cold exposed domestic ruminants is discussed.

Methods

Animals, Preparation and Treatment

A detailed description of the methodology in the present experiment has been previously described (chapter 4). Twelve crossbreed wethers, six-months of age and weighing 35-50kg were used. At the start of the study the sheep were treated for internal and external parasites and recieved injections of retinol, cholecalciferol, and alpha-tocopherol. All animals were shorn at two week intervals to a constant fleece depth of about 1 cm. The sheep were housed in individual metabolic crates in climatically controlled rooms under constant lighting. Carotid skin loops were established on the left carotid artery of each sheep following the procedures described by Hecker (1974).

Diet

A near maintenance diet was provided, consisting of 1650g (air dry) of brome alfalfa pellets (Bromus inermus, Medicago sativa) offered in 825g servings at 0800h and 1700h daily. (D.M.=91.65%, A.D.F.=49.99%, energy=4.493 kcal/g, protein=14.23%). The ration was ground through a 5mm screen before pelleting to 3 mm diameter. Free access to water and iodized block salt was also provided.

Thermal Treatments

The sheep were randomly assigned to three treatment groups , the treatments being:

Control (CON) 10-12 weeks exposure to 18.7 (range 13.3-22.2)C db 11.6 (range 10.5-16.1)C wb

Acute Cold (AC) Conditioning to 18C db, 11C wb for six weeks, followed by 12h exposure to 3 (range 1.6-4.4)C db 0 (range -1.6-2.2)C wb

Chronic Cold (CC) 10-12 weeks exposure to 3.3 (range 1.6-4.4)C db, 0 (range -1.6-2.2)C wb.

Catheterization

Catheter placement into the left ventricle and right atria as described by Hales (1973b) and chapter 6 of this text, was completed using surgical Tygon Microtubing 0.04-0.07mm I.D. (Norton Plastics, Akron, Ohio, U. S. A.), utilizing the previously prepared carotid skin loops. Catheter placement was accomplished with the aid of a pressure transducer and verified upon anatomical dissection and tissue radionuclide counting.

Microsphere Injections

Radioactive microspheres (15μm diameter) containing the nuclides Ce-141 and Sn-113 in doses of approximately 0.22mci were used in the experiment. The microspheres were contained in 10 ml of 10% dextran with a trace of Tween 80 suspending agent. Plastic syringes (20ml) were used to inject the spheres over timed intervals of 60 to 90 seconds. Withdrawl of blood samples for the calculation of cardiac output (CO) and arterial venous anastomosis (AVA) measurements were also into plastic syringes (50cc) and taken over timed intervals of 120 to 180 seconds. Withdrawal of samples at a rate of 15.7 ml/minute were started simultaneously with the injection of the microspheres.

Tissue Counting

Following the injection of the microspheres, the animals were subsequently terminated using an overdose of sodium-pentabarbital I. V.. All organs or tissues were dissected within 24 hr and counted in one of two large volume nuclide counters utilizing 7.5 mm by 7.5 mm NaI crystals, with an Ortec #456 high voltage supply, Canberra amplifier #1417B and Canberra scaler #1437. The wet weights of organs or tissues were obtained at the time of dissection.

Ration Digestibility

During a ten day period just prior to the microsphere injections, a total collection of feces was carried out daily between 0900 hr and 1200 hr on the CON and CC animals. The samples were analysed for percent moisture, nitrogen, fiber, and energy, from which estimates of digestibility for dry matter, protein, acid detergent fiber, and energy were obtained.

Respiratory Measurements

All respiratory gas analysis was done using a Beckman gas analyser (Beckman Instruments Fullerton, California), in conjunction with an open air circuit respiratory hood, as has been described by Young etal. (1974).

Statistical Analysis

Significant differences between means for treatment groups were evaluated by analysis of variance and Student-Newman-Keules multiple range test (Steel and Torrie, 1960).

Results

Cardiorespiratory and Metabolic Rate

Detailed information regarding cardiorespiratory and metabolic values for the three treatment groups is given in Table 5. Primarily, both acute and chronic cold exposure caused a 20-30% increase in oxygen consumption, or metabolic rate, and a 10-15% increase in cardiac output, although cardiac output was not significantly different at the $P<0.05$ level. Chronic cold exposure also caused a 17% increase in heart rate, while a decrease in respiratory rate was seen in both the acute and chronic cold exposed sheep.

Digestibility

The effect of cold temperature on digestibility of dry matter, energy, nitrogen, and acid detergent fiber are shown in Table 5. Cold exposure reduced the digestibility of these feed components up to 0.08% per degree C.

Blood Flow and Blood Flow Distribution

As illustrated in Table 6, cold exposure caused a 22% and 27% reduction in the percent of cardiac output (CO)being distributed to the gastrointestinal tract in the acute and chronic cold exposed animals respectively; the main reduction occurred in the reticulo-rumen.

When expressed as ml of flow/100g tissue/minute, Table 6, the blood flow to the total GIT was not significantly different among the treatment groups. However, the specific reduction in blood flow (ml/100g/min) was still evident in the reticulo-rumen in both cold groups measured as a 51% and 39% reduction in reticulo-rumen blood flow in the AC and CC sheep respectively. The relative blood flow values (Table 7) also support these trends and furthermore demonstrate the blood flow in the various GIT tissues in general to be between 2 to 8 times the body average.

Discussion

Cardiorespiratory and Metabolic Measurements

Cold exposure in the present study precipitated cardiorespiratory and metabolic physiological changes including an increased oxygen consumption, or metabolic rate, an increased heart rate, and a reduced respiratory rate. These changes have also been observed in similar studies with cold exposed sheep, (Westra and Christopherson, 1976; Christopherson, 1976; Hales etal. 1977) and seem to be representative of a consistent adaptive response to cold stress in these domestic ruminants.

Digestive function and Blood Flow

A reduced digestibility in cold exposed sheep has been documented by Westra and Christopherson (1976), as well as in other recent studies (Kennedy etal, 1977; Kennedy and Milligan, 1978). The digestibility results of the present study follow this same pattern and seem indicative of digestive functional changes occurring in these animals as a result of cold exposure. However, the magnitude of reduction was less in the present study.

Furthermore, Westra and Christopherson (1976) have demonstrated that cold exposure can induce an increased reticulo-rumen motility and an increased rate of passage of digesta, possibly mediated by thyroid hormones, which consequently reduces the retention time of digesta in the reticulo-rumen. These observations would suggest, as is supported by Kennedy and Milligan's study (1978), a greater concentration of less digested or nutritively more complete digesta being passed to the intestinal sections of the GIT.

Kennedy and Milligan (1978) have demonstrated that in cold exposed sheep, the amount of organic matter and non-ammonium nitrogen digested in the intestines was higher than in warm exposed or control sheep. They further report that the increased digestion in the intestines of the cold exposed sheep compensated, to a degree, for the reduction of dry matter, organic matter, and cell wall constituents digested in the reticulo-rumen. Kennedy and Milligan (1978) also suggested that blood flow to the absorptive sites in the rumen wall in sheep may be one factor affecting urea transfer measurements between sheep given two different levels of feed intake in their study. This may be especially relevant considering that on a tissue basis it has been pointed out by Hoffman (1973) and Engelhardt and Hales (1977) that GIT mucosa in particular, because of its vascular supply, seems to have a prime involvement and importance in the ruminant in terms of nutrient absorption and metabolism. The role of stomach blood flow and environmentally influenced blood flow shifts also has been discussed by Dobson etal. (1976a) as being influential in terms of affecting nutrient flow, and can be a limiting factor in substrate exchange across the gastrointestinal epithelial membranes.

In the present study, the increased nutrient and thermal demand of non-digestive organs and tissues in the acute and chronic cold animals, as a compensating thermo-regulatory response to their environment is likely the basic cause of reduced cardiac distribution to the GIT. However, of the blood distributed to the GIT, the present study has demonstrated that the cold exposed sheep show a proportionately reduced blood flow to the reticulo-rumen, and an enhanced blood flow emphasis to the intestinal regions. The reduced reticulo-rumen blood flow seen in the present experiment is possibly the result of increased reticulo-rumen blood shunting.

According to studies such as those by Renkin (1968), Engelhardt (1970), and Dobson etal. (1976a), capillary blood flow affects the absorption of nutrients from the GIT. The present study has demonstrated a reduction of GIT capillary blood flow, emphasizing reduced reticulorumen yet maintained intestinal blood flow as a result of cold exposure. Consequently, by influencing nutrient absorption, this altered or redistributed GIT blood flow emphasis in cold exposed sheep is perhaps, at least in part, responsible for the reported digestive functional differences.

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NOTE: References for this chapter are contained in the thesis bibliography.

Table 5: Body weight, intake, digestibility, cardiorespiratory, and metabolic measurements in sheep exposed to near themoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures

	Treatment			
Intake (g/day)	CON	AC	CC	SEM
number of animals	4	4	4	
water (end of treatment)	3200	1900	2750	
feed(air dry) dry matter	1512	1512	1512	
protein	234.8	234.8	234.8	
Digestability (%) *				
dry matter	54.03		52.84	1.28
energy	54.29		53.35	1.23
protein	63.98		63.68	1.36
acid detergent fiber	51.06		50.12	1.44
body weight(kg) (end of treatment)	41	45	40	3.75
Heart rate/min	131^{e}	120^{e}	157^{d}	12.2
Respiration rate/min	73^{a}	22^{b}	22^{b}	7.0
Cardiac output (liters/min)	5.5	6.5	6.1	0.79
Hematocrit %	25.3^{e}	32.3^{d}	25.8^{e}	2.2
Oxygen consumption (ml/min)	316.18^{b}	465.65^{a}	397.85^{a}	35.47
Mean Body Surface Temperatures (C)				
Trunk	34.6^{a}		31.2^{b}	1.23*
Extremities	33.1^{a}		20.6^{b}	2.11*

$^{a,\ b,\ c}$ Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

$^{d,\ e,\ f}$ at $P<0.10$ probability levels.

* Statistically compared using Students paired T test.

SEM is the standard error of treatment means.

Table 6: Percent distribution of cardiac output and blood flow/100 g/min to the gastrointestinal tract of sheep exposed to near themoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures

Tissue	Percent Distribution				Blood Flow/100g/min			
	Treatment				Treatment			
	CON	AC	CC	STANDARD ERROR	CON	AC	CC	SEM
Reticulorumen	6.95^{d}	3.36^{e}	4.21^{e}	0.536	56.04^{d}	33.95^{e}	37.76^{e}	6.41
Omasum	1.63	1.17	1.06	0.174	69.94	57.80	54.10	9.32
Abomasum	3.56	3.61	3.13	0.550	88.1^{ed}	136.0^{d}	73.2^{e}	17.89
Small Intestine	8.55	7.71	6.70	0.867	71.2	83.3	50.1	10.35
Large Intestine	5.70	4.63	4.25	0.438	63.27	44.76	53.13	7.21
Total	26.4^{a}	20.5^{b}	19.4^{b}	2.12				

Large intestine includes section from ilio-cecal junction to rectum, including colon.

$^{a, b, c}$ Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts

$^{d, e, f}$ at $P<0.10$ probability levels.

SEM is the standard error of treatment means.

Table 7: Percent distribution of gastrointestinal blood flow and relative blood flow in sheep exposed to near thermoneutral (CON), acute cold (AC) and chronic cold (CC) temperatures

	Percent Distribution of GIT Blood Flow Treatment				Relative Blood Flow Treatment			
Tissue	CON	AC	CC	STANDARD ERROR	CON	AC	CC	SEM
Reticulorumen	26.42^{a}	16.02^{c}	21.61^{b}	1.6	4.00^{a}	2.17^{b}	2.45^{b}	0.36
Omasum	5.59	5.93	5.48	0.8	5.04	3.86	3.54	0.60
Abomasum	13.77	17.75	16.29	1.9	6.36	8.84	4.72	1.06
Small Intestine	33.36^{b}	37.77^{a}	34.64^{b}	1.6	5.17^{d}	5.47^{d}	3.24^{e}	0.61
Large Intestine	20.86	22.53	21.98	1.5	4.53^{a}	2.88^{b}	3.48^{ab}	0.42
	100	100	100					

a, b, c Means with different superscripts within rows are statistically different at $P<0.05$ and superscripts d, e, f at $P<0.10$ probability levels.

SEM is the standard error of treatment means.

E. CHAPTER 6 : GENERAL DISCUSSION

A discussion and interpretation of the various circulatory and metabolic changes occurring in shorn sheep in the current study as a result of acute and chronic cold exposure are presented in chapters 4 and 5 . The purpose of the present chapter is to expand on the discussion in these chapters, and to further hypothesize on the significance of the data obtained in the study.

6. 1 General Effects of Cold Exposure

The general effects of cold on mammals was given and several characteristic physiological responses were reviewed in chapter 2. The cold exposed animals in the present experiment demonstrated many of these characteristic responses.

A 20-30% increase in oxygen consumption and a 70% decrease in respiration rates were observed in the AC and CC sheep as well as a 10-15% increase in cardiac output, (although cardiac output failed to reach significance at the 0.05 level). A 17% increase in heart rate was also measured in the CC animals. An increase in metabolic activity of the thyroid gland may be inferred from the 95-100% increase in blood flow to this gland demonstrated in the AC and CC animals, and an increase in adrenal gland activity could be inferred from the 31% increase in adrenal gland blood flow in the AC sheep. Although a reduced peripheral skin blood flow was not detectable by the microsphere measurements in the present study, reduced surface skin temperatures as well as reduced blood flow to the radius and ulna and hoove tissues was detected, hence, indicating a degree of peripheral vasoconstriction.

In general, these physiological responses indicate that these animals responded in a characteristic manner to the cold treatments.

6. 2 Skeletal Muscle

The present study has clearly illustrated an increase in blood flow to the shivering skeletal muscles as a result of both acute and chronic cold exposure. This was observed in the AC animals whereby a 2 and 4 fold increase in blood flow respectively to the gluteus maximus and triceps was measured. These increases were even more pronounced in the CC animals where a 5 to 15 fold increase in blood flow to the gluteus maximus and triceps respectively was measured. As mentioned in chapter 4 this observation has also been reported by Hales (1976a) and was suggested by Hales to be due to the increased energy or nutrient demand of the shivering muscle in acute cold exposed sheep. The suggestion that skeletal muscle may be important as a heat producing tissue in chronic cold exposed animals as well as acute cold exposed animals has been previously hypothesized by other researchers (Webster, 1975b; Jansky, 1976; Foster etal.

1978). Foster and Frydman (1978) have recently demonstrated the involvement of skeletal muscle in thermogenic reactions in cold acclimated rats. In the present experiment, the increased blood supply to the skeletal muscle of the chronic cold exposed sheep is suggestive of non-shivering thermogenesis in these tissues in sheep. Feasibly, the observed increase in blood flow to skeletal muscles in AC and CC exposed sheep facilitated not only nutrient demands of the muscle, but also heat removal or exchange for the maintenance of homeothermy. In the present experiment, between 5-6% of cardiac output in the CON sheep versus up to 30% of the cardiac output in the CC sheep was distributed to the skeletal muscle, (based on a calculation of skeletal muscle equal to 27% of body mass; Hales 1976b). Even considering that all muscles may not have the same flow rate as those particular muscles measured in the present experiment, nor may all muscles be involved in shivering and nonshivering thermogenesis, this nevertheless suggests the importance of this tissue to these cold exposed animals.

6. 3 Internal Organs and Glands

The increase in blood flow to the thyroids and adrenals of the cold exposed sheep was the main alteration in blood flow seen in the internal organs and glands. An increase in metabolic activity in these tissues and hence an increased output of thyroid and adrenal hormones could perhaps be concluded. According to the comments put forward by Webster

(1974) the significance of increases in thyroid and adrenal hormones, as a result of cold exposure, is still receiving much speculation. However, catecholamines, in conjunction with a "mediating" effect of thyroid hormones, do seem to play a role in cold induced thermogenesis via an influence on cell membrane function and volatile fatty acid metabolism (Hardy, 1961; Sellers etal. 1971; Webster, 1974). Consequently, an increase in adrenal and thyroid gland activity, indicated by an increased nutrient blood flow, as seen in the present experiment, may be a reasonable consequence of cold exposure. However, a direct thermogenic effect induced by catecholamines has yet to be proven in sheep (Webster etal. 1969).

6. 4 Skin and Fat

The mobilization of fat or fatty acids in sheep as a result of cold exposure has been reported (Alexander, 1972; 1973; Hales etal. 1976a; Thompson etal. 1978). The vasoconstriction of the extemities and skin vasculature functioning as a heat conservation mechanism has also been reported (Alexander, 1973; Hales etal. 1976a). The involvement of these mechanisms as a cold response is seen to be of less magnitude in the present study. As mentioned in chapter 4, the cause of this difference between the present experiment and similar cold studies on sheep is perhaps owing to differences in exposure temperature and in the age and size of the animals concerned. Also, the fact that the cutaneous skin blood flow was not differentiated from the subcutanious blood flow may have masked any reduction in cutaneous blood flow.

6. 5 Respiratory System

The increased metabolism in cold stressed sheep necessitates an increase in oxygen consumption (Kleiber 1975). However, increased oxygen consumption does not seem to be synonymous with increased respiration rate, as can be seen in the present experiment and also by Hales (1976a) observations. The increased oxygen consumption in the present experiment is possibly due to an increased alveoli absorption time, and hence increased oxygen extraction, feasibly enhanced also by an increased red blood cell count. The present study illustrated a 22% and 2% increase in hematocrit in the AC and CC animals respectively. Although only the 22% increase was significant at the $P<0.05$ level.

The current reduction in ventilation rate as well as the reduced lung (bronchial artery) flow in the cold exposed sheep is perhaps effective in reducing conductive heat loss from the upper respiratory tract. However, lung blood flow measurements can be biased by AVA flow and should be viewed in light of this blood shunting. As discussed in chapter 4, this may especially be relevant in heat stressed sheep where AVA blood flow can be of considerable magnitude (Hales, 1973a).

6. 6 Gastrointestinal Tract

A redistribution of blood within the GIT of cold exposed sheep has been demonstrated in the present experiment. The effects of this redistribution are discussed in chapter 5, however, the reasons for the redistribution seem to be unclear.

Blood flow is responsive to the nutrient and thermal demands of the animal. Consequently, GIT blood flow redistribution would be the effect, rather than the cause of physiological stimuli inducing this redistribution. The question then becomes what factors are causing the redistribution?

The existence of neural, mechanical, and chemo-receptors affecting the flow of digesta in the GIT of ruminants has been demonstrated and is discussed in detail by Iggo and Leek (1970). However, a simultaneous stimulation of GIT blood flow by these particular factors has not yet been documented .

Some evidence exists supporting the operation of either neural and/or chemical receptors as blood flow mediators. Duncan (1953) has demonstrated that severing the vagal nerve supply at the diaphragm in sheep will cause a stoppage of motility in the GIT, hence, indicating a possible neural control system in the GIT. The possible role of catacholamines in GIT blood flow control may as well be reasonable. Dobson and Phillipson (1956) and Garbulinski (1973) have demonstrated that GIT vasculature is responsive to vasoactive agents including adrenalin. Although there is little or no evidence of an elaborate nervous supply to the epithelium or sub-epithelium cell layers of the GIT (Steven and Marshall, 1970) the existence of dopamine containing cells in the intestines of sheep has been shown (Atkins etal. 1971). Consequently, these reported literature findings would suggest that catecholamines, or other vasoactive agents, may have an influence on GIT blood flow in ruminants.

Further speculation as to the cause of blood flow redistribution in the GIT of cold exposed sheep would possibly suggest the involvement of digestive end products, or Krebs intermediate metabolites, including acetate, citrate, fumarate, maleate, alpha-ketoglutarate, oxaloacetate, and succinate. The influence of digesta or digestive end products such as acetate, propionate, butyrate, and cabon dioxide on GIT blood flow is discussed by Dobson and Phillipson (1956) and was demonstrated by Sellers etal. (1964). Giachetti etal. (1977) also refers to vasoactive intestinal polypeptide as playing a role as a vasoactive transmitter or modulator of synaptic function. However, the specific influence of this agent in GIT blood flow in cold exposed sheep is not determined. The influence of volatile fatty acids on portal blood flow as a result of cold exposure is also reported by Thompson etal. (1975; 1978). Consequently, digestive end products do apparently influence GIT blood flow. However, determining what specific end products may be involved in influencing GIT blood flow and if they work in conjunction with such other factors as microbial and GIT tissue metabolism effects on a local level remains to be investigated.

The fact that the GIT accounts for 20-25% of the cardiac output signifies the importance of this tissue, and in particular, the mucosa or absorptive tissue layers, under any thermal environment. Any factors, environmental or otherwise that reduce or adversely affect this obligatory blood flow to the GIT would have a significant detrimental influence on nutrient attainment in the animal.

F. CHAPTER 7: SUMMARY AND CONCLUSIONS

The present study demonstrated changes in blood flow distribution and other cardiovascular and metabolic parameters as a result of both acute (AC) and chronic cold (CC) exposure of shorn sheep. The most significant aspects demonstrated by this study can be summarized as follows:

(1) Increased blood flow to the thyroids of 100% and 95% in the AC and CC animals respectively and a 31% increase in blood flow to the adrenals of the AC sheep is indicative of the metabolic significance of these tissues during cold exposure. However, the precise mechanism of thyroid and adrenal hormonal action in the cold exposed sheep is as yet uncertain.

(2) A two to four fold increase in blood flow to the gluteus maximus and tricep skeletal muscle in the AC sheep and a five to fourteen fold increase in blood flow to these respective muscles in the CC sheep suggests the involvement of skeletal muscle in cold induced thermogenesis.

(3) The present study has demonstrated a cold induced redistribution of GIT blood flow showing a reduced reticulo-rumen, and maintained intestinal blood flow emphasis. Although the reasons are unclear at this time, the decreased reticulo-rumen blood flow seems not to be just a temporary "cold compensation" mechanism as was proven by the response of the CC sheep. The blood flow changes in the

gastrointestinal tract are likely influential in digestive functional changes observed in cold exposed ruminants.

(4) The reasons for or causes of many of these observed cold induced blood flow redistributions, although often speculated, remain to be proven.

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APPENDIX

H. APPENDIX I : MATERIALS AND METHODS (SUPPLEMENTARY)

The methods sections of chapters 4 and 5 contain a description of animal use, measurement techniques, and analysis procedures which are more appropriate for the inclusion in a paper for publication in a scientific journal. This appendix primarily contains supplementary details of the methodology and procedures used in the experiment and not included in the papers (chapters 4 and 5) or, where more detail was considered appropriate for thesis consideration.

I. 1 Microsphere Examination and Handling

The radioactive microspheres used in the present experiment were obtained from New England Nuclear, Boston, Massachusetts. The spheres were shipped in 10 ml volumes of 1 millicurie (mci) each, contained in 10% dextran with 0.01% Tween-80 suspending agent. An aliquot of the shipping medium was drawn off and assayed for radioactivity as a possible indication of nuclide leaching in the spheres. No "above background" radioactivity in the shipping solution was found. The spheres were stored in lead shielding until the day of injection at which time they were made up to a volume of 45 ml by the addition of 10% dextran. This solution was homogenously mixed with an ultrasonic mixer (type Sonic 300 Dismembrator, Artec Systems Corp., Farmingdale, N. Y.) and approximately 10 g of solution was then weighed out into 20 ml tared plastic syringes for injection into the sheep.

The physical diameter of the microspheres used in the present study was guaranteed by the supplier (New England Nuclear) to be 14.6±1.0μm, for the cerium, and 15.1±1.1μm, for the tin. Microscopic examination of a representative sample of the spheres with a micrometer grid demonstrated that the average diameter was of 15.08μm with a range of 14.7 to 15.5μm. However, 98% of the spheres measured were within .3μm of the average. The mean cerium and tin diameter values were not statistically different from each other (15.09μm for cerium, 15.08μm for tin) and later analysis showed no statistical differences between nuclides in terms of distribution within a tissue.

I. 2 Injection Solution and Solution Additives

In the present study the microspheres were contained in a solution of 10% dextran with 0.01% Tween 80 suspending agent. Foster etal. (1977) reported that dextran was not used in their experiment to retard the settling of microspheres because of reported anaphylactic reactions to dextran in rats. It is also reported by Goodman and Gillman (1975), that when used as a plasma extender, dextran can cause an antigen reaction and sensitivity in some people. However, in the present study, no anaphylactic reactions were observed to either dextran or the Tween agent in the sheep, nor have any such reactions been reported in the literature with respect to sheep. This point may warrant further research specifically with regards to ovin sensitivity to dextran or Tween agents.

I. 3 Infusate Temperature

Bligh (1963) discusses several experiments in which cold saline injected into the vena cava reduced or suppressed panting in sheep. He concluded the existence of thermal receptor sites in the venous trunk. Consequently, it might be inferred that the injection of a room temperature microsphere solution into the left ventricle could affect thermal regulation in the animal and hence the distribution of blood. This factor is seldom mentioned in the methods descriptions of microsphere experiments discussed in the literature.

In the present experiment measurements on additional sheep showed that approximately 50cc of room temperature saline was required to be injected into the left ventricle, within a five minute period before any noticeable changes occurred in thermoregulation (shivering or postural movements). Consequently, considering that the microspheres in the present experiment were contained in only 10cc of room temperature saline, we assumed that the injection quantities used in this study would not affect thermoregulation. However, the effect of injectate temperature should be considered in interpreting microsphere data where it is potentially an influential factor.

I. 4 Body Positioning During Microsphere Injection

Pre-experimental tests, showed that an animal's body movement could influence its blood pressure when measured via carotid artery catheters. For example, movement or exercise within the metabolic crate increased blood pressure by increasing heart rate and cardiac output. Conversely, if the animal layed down, blood pressure was reduced due to a reduction in heart rate and cardiac output. An altered blood pressure could influence the distribution of the microspheres, hence biasing the treatment or temperature effect on blood distribution. Consequently, all animals were trained several weeks in advance of the microsphere injections to stand in a relatively constant body position, by haltering them to a bar at the front of their metabolic crates. Halter training sessions were maintained for about one half hour to one hour, or approximately the same time period required to inject the microspheres and to take the associated measurements described in Figure 1. A consistent alignment of the head neck and trunk was consequently obtained.

I. 5 Behavioural Considerations

Human movement within the animal rooms appeared to affect the behaviour of the sheep. This was probably due to the animals psychological conditioning to feeding and handling by humans. This induced behaviour could influence blood flow in the animals by affecting catecholamine release and hence influencing blood pressure. Therefore, where required, the animals were screened off from the equipment area and also were subjected to a low volume radio background noise, for several weeks in advance of the microsphere injections. Consequently, because all sampling and measurements were made from outside the animal room and because the animals were given a 20 to 30 minute "quiet period" prior to experimentation, the noise and physical disturbance caused by the researchers appeared to have little influence on the sheep.

I. 6 Equipment

The technical description of the equipment used in the present study is given in the following listing. Additional discussion of equipment and methods of equipment use are also given in chapters 4 and 5.

(1) Temperature recorder , model BAT-8, Bailey Instruments Inc., Saddle Brook, N. J., USA; used for the measurement of body and surface temperatures.

(2) Drying oven, Despatch Oven Comp., style V-31, The Partlow Corp., New Hartford, N. Y., USA; used for obtaining tissue dry weights.

(3) Infusion/withdrawal pump, Harvard Apparatus, 150 Dover Rd., Mills M. A., USA; used for the withdrawal of blood samples for CO and AVA estimates.

(4) Respiratory Gas Analyser, Beckman Instruments, Fullerton California USA, Printer model, Honeywell. Ft. Washington, P.

A.. CO2 anolyser model 215A S/N; used for the measurement of respiratory gas.

(5) Sanborn Physiological Recorder, Carrier pre-amplifier 350-1100C, high gain pre-amplifier 350-2700C, recorder 7700 series. Hewlett Packard, Watham, Mass. USA; used for recording heart rate, and blood pressure traces.

(6) Pressure Transducer, Model 267BC, Hewlett-Packard, Waltham Mass. USA; used for measuring blood pressure.

(7) Blood Gas Analyser, Blood Micro System type BMS 3a, Acid Base Analyser, PHM71. Radiometer/Copenhagen, Denmark; used for measuring blood pH, pCO2, and pO2.

(8) Tygon Microbore Tubing, Formulation S-54-HL, I. D. 0.04-0.07 mm. Surgical and hospital use, Norton Plastics and Synthetics Division , Akron, Ohio, USA; used for injection and withdrawal catheters.

(9) Isotope Counters, NaI crystals, 7.5 cm. Ortec high voltage supply # 456, Ortec Inc., Oak Ridge, Tennessee, USA., Canberra amplifier # 1417B, Canberra Industries Inc., Meriden, Connecticut, USA., Canberra scaler # 1437; used for radioactivity counting.

(10) Ultrasonic Mixer, Sonic 300 Dismembrator, Arteck Systems, Corp. Farmingdale, N. Y. USA; used in the preparation and mixing of microsphere solutions.

I. 7 Respiratory Gas Analysis and Respiration Rates

I. 7. 1 Respiratory Gas Analysis

The rate of oxygen consumption in the sheep was measured in an open circuit respiratory gas analysis system (Young etal. 1974). Respiratory gas was collected via a hood enclosing the animal's head. This method of respiratory gas collection was preferred because it disturbed or emotionally upset the animal less than the available alternative of a more restrictive face mask. Oxygen consumption was used to calculate metabolic heat production assuming a caloric value for oxygen of 4.89 Kcal/liter.

I. 7. 2 Respiratory Rate

Respiratory rate was measured in the present experiment with the use of a tambour recording device. This system involved essentially placing a flexible, convoluted 2.5 cm rubber tubing around the thorax of the sheep. Consequent breathing action moving the rib cage and diaphragm would, hence, cause distention and contraction of the tubular belt. By connecting this belt to a flexible 0.5 cm I. D. tubing, which was in turn connected to a manometer, respiration rate could be monitored by deflections in the manometer.

I. 8 Blood Analysis

Following the injection of the microspheres, two separate 10 ml samples of blood were taken from the carotid artery, one into a heparinized syringe, the other into a non heparinized syringe. The blood samples were immediately transferred to glass test tubes. From the heparinized blood sample, duplicate aliquots were drawn into capillary tubes for hematocrit determination as described in chapter 4. The test tube containing heparinized whole blood was then sealed with parafilm (parafilm "M" American Can Company Wisconsin, USA) and frozen at -5 to -10 C for later use in hemoglobin analysis. The unheparinized blood was allowed to clot at room temperature and was then centrifuged at approximately 3000-5000 rpm. in a GLC-1 General Lab centrifuge for 10 to 15 min.. The serum was drawn off into a glass test tube, sealed with parafilm, and frozen for later thyroxin (T4) analysis.

At the same time as the above blood samples were taken, additional 2 ml blood samples were obtained from the carotid artery and right atria with heparinized 5 ml plastic syringes. The needle ends of the syringes were then sealed by inserting them into a rubber stopper. These two samples were immeadiately used to measure blood gases (pO2, pCO2) and pH (Blood Gas Analyser, Blood Micro System, type BMS 3a).

Values for T4 were determined by the method described by Chopra (1972); T4 antibody was purchased from Calbiochem-Behring Corp. LaJolla, California. Hemoglobin concentration was determined according to the method of Wong (1954).

I. 9 Body Surface Temperatures

Temperature values for the flank, midside, dorsal midline, ears, and lower front leg of the CON and CC sheep, were taken with thermocouples and temperature recorder (model Bat-8, Bailey Instruments, Saddle Brook, USA). Each thermocouple was placed on the skin surface, and held in place until a constant temperature readout was obtained. The thermocouple wires were insulated from the operator's hand heat by a synthetic foam covering.

I. 10 Dissection

After being killed with an overdose of sodium pentabarbital (Nembutal) the carcasses were placed in a room kept at 3C db. They were moved to a larger dissection room within 24 hr and placed on a stainless steel dissection table. Dissection was carried out primarilly by two technicians, each generally responsible for dissecting specific body areas. A third technician was responsible for weighing, recording, and storing of tissue samples. Tissue and organ samples were sealed in separate containers and then frozen at -5 to -10 C until tissue nuclide counting was done. Whole organs such as liver, heart, and gastrointestinal organs were placed in cylindrical containers measuring approximately 5cm by 8cm. Smaller organs and tissues such as adrenals, thyroids, and skin tissue were placed in smaller (4cm by 6cm) cylindrical plastic containers. Blood samples (collected for cardiac output (CO) and arterial venous anastomoses (AVA) estimates) were transferred to 50cc glass beakers for gamma counting. A fourth person (the author) coordinated and supervised the dissection procedures with regard to decisions on sampling sites, and storage of samples. Care was taken during dissection not to disturb the injection and sampling catheters until the catheter position had been photographed and verified by internal examination of the heart.

The tissue wet weights reported in the appendix were obtained at the time of dissection. Dry weights were obtained after nuclide counting was completed by either freeze drying or oven drying the samples to a constant weight. Tissue nuclide counts were corrected for half life decay to the day of nuclide injection using the formula ;

Activity = (activity at time zero) $(0.5)^{t/t.5}$,

where t = days, or parts thereof since injection

and t.5 = the half life of the nuclides (32.5 days for Ce-141 and 115 days for Sn-113).

The injectate cpm were also corrected to the day of each injection; consequently , all tissue counts could be compared directly with injectate counts.

I. 11 Istotope Counting

Two gamma emitting nuclides Ce-141 and Sn-113 were used in the present experiment. The cerium and tin nuclides were two of the most compatible tracers available in terms of energy separation , spillover, and half life accordance. Isotope choice was also based on the limitations and resolution capability of the counting system available for the study.

When measuring the radioactivity of an nuclide, a random scattering of the emitted radioactive particles occurs and as such, collecting or measuring all of the emitted activity. The percentage of nuclide that is measured, which varies according to the type of equipment used, is termed the counting efficiency.

In the present experiment, two large volume radionuclide counters were used (see section I.6 for equipment description). A description of this equipment is given by Lyster (1971). The use of a large volume counting system and whole organ counting was expected to ensure more reliability than more commonly used subsampling or representative sampling procedures. The measurement of counting accuracy and efficiency in the counters were estimated by placing in the counters an "nuclide source" container similar to the containers used for tissue collection, containing a known amount of either Ce-141 or Sn-113 nuclide. Consequently, by comparing the counts per minute (cpm) obtained with the actual amounts of nuclide in the containers, the efficiency value could be estimated.

The nuclide counter used to measure all the tissues from the AC sheep (counter#1)demonstrated counting efficiencies shown in Figure 2. Use of this efficiency curve facilitated the adjustment of nuclide counts to allow for geometric differences in the different tissues. Katz and Blantz (1972), felt that the establishment of an efficiency curve was essential when using counting procedures of this nature in order to eliminate counting biases due to tissue height differences.

Isotope counter number 2 used to measure the CON and CC sheep tissues utilized dual detection crystals and demonstrated uniform counting efficiencies of 4.59% and 3.01% for Ce-141 and Sn-113 respectively.

When using two nuclides, due to the width of the photo peak or peaks, there is often an overlapping or interference of one nuclide with the other. This phenomena is known as "spillover". In the present experiment, essentially no cerium spillover occurred into the tin "window", however, the tin, being of higher energy, did spillover into the cerium "window". This spillover was measured as the percent of cpm in a mixture of cerium and tin that was in addition to the cpm contributed by a single nuclide when measured in one of the nuclides "counting windows". The spillover values for counter number 1 are shown in Figure 3. The spillover value of Sn into Ce for counter number 2 was constant at 22.2%.

I. 12 Calculations

The proportion of systemic output distributed to an organ can be estimated with the radioactive microsphere technique by comparing the number of microspheres (or radioactivity) in that organ to the total number of microspheres (or radioactivity) injected. However, an actual quantitative determination of flow rate to an organ can also be determined. Hales (1973) and Heymann etal. (1978) describe various methods for calculating cardiac output using radioactive microspheres. The most common method, and the one used in the present study, is that of a "surrogate organ flow", or what is often termed a "reference sample technique", or "artificial organ technique". This method involves the withdrawal of blood from an artery at a constant rate via a plastic syringe. The withdrawal of blood is started simultaniously or within 5 seconds of the microsphere injection, and continued for approximately one minute after the injection of microspheres. Consequently, the withdrawal syringe or "artificial organ" will contain a proportion of the injected microspheres. Assuming that the flow rate or withdrawal rate in the artificial organ is proportional to the cardiac output, just as the radioactivity in the artificial organ is proportional to the injected radioactivity, then cardiac output can be calculated using the formula described in the following paragraphs.

This same reasoning can be used in estimating the arterial venous anastomosis (AVA) flow, which is also ilustrated in the following paragraphs. However, AVA flow estimated in this way provides only an overall or total body estimate of AVA flow. This gives no estimate, for example, of the mesentary AVA flow which, if significant, could bias the radioactivity (or number of microspheres) attributed to the liver capillary flow.

A description of the calculation methods used for cardiac output (CO) and arterial venous anastomosis (AVA) estimates is given in the accompanying examples. The terms used in these calculations are defined as follows: "syringe right heart" or "right atria" refers to the mixed venous blood sample taken from the right atria which was used in AVA calculations. "Syringe carotid artery" or the "artificial organ" blood sample refers to the blood sample taken from the left exteriorized carotid artery for calculations of cardiac output.

The calculations of cardiac output and arterial venous anastomosis were as follows;

(1) Cardiac output (CO) = (F) (cpm D')/d' in ml/min.

Where, F = sampling rate in the artificial organ (carotid artery) in ml/min.

Where, D'= injectate cpm

Where, d'= cpm in artificial organ withdrawal sample.

example: F= 16.8 ml/min, D'= 25995281, d'= 79695

therefore, C O= (16.8) (25995281)/79695 = 5479.9 ml/min.

(2) Arterial Venous Anastomosis (percent of CO through AVA)

AVA= (d''/D') (CO/p) (100)

d''= cpm in the withdrawal sample from the right atria

p= flow (ml/min) or withdrawal rate for AVA sample.

Figure 2: Relationship between radionuclide counting efficiency and sample volume of counter #1 calculated with known quantities of Ce-141 and Sn-113 radionuclide source

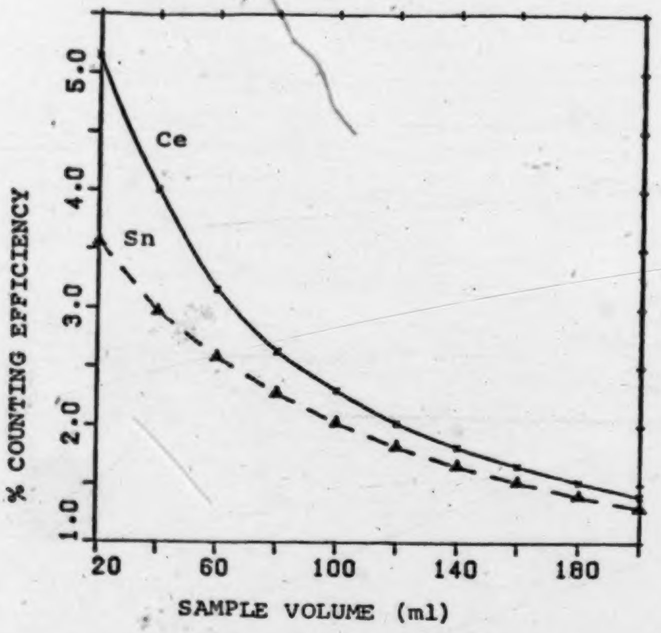
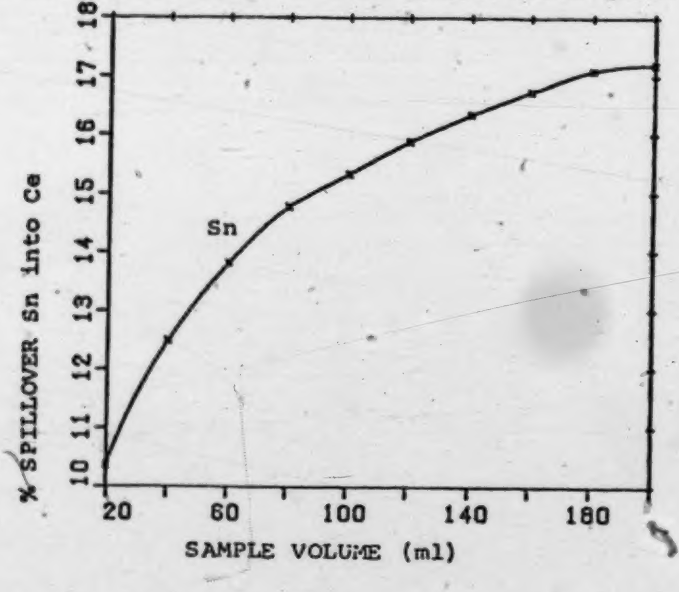


Figure 3: Relationship between percent "spillover" of Sn-113 radionuclide into the Ce-141 "counting window" and sample volume of counter #1 calculated with a known quantity of Sn-113



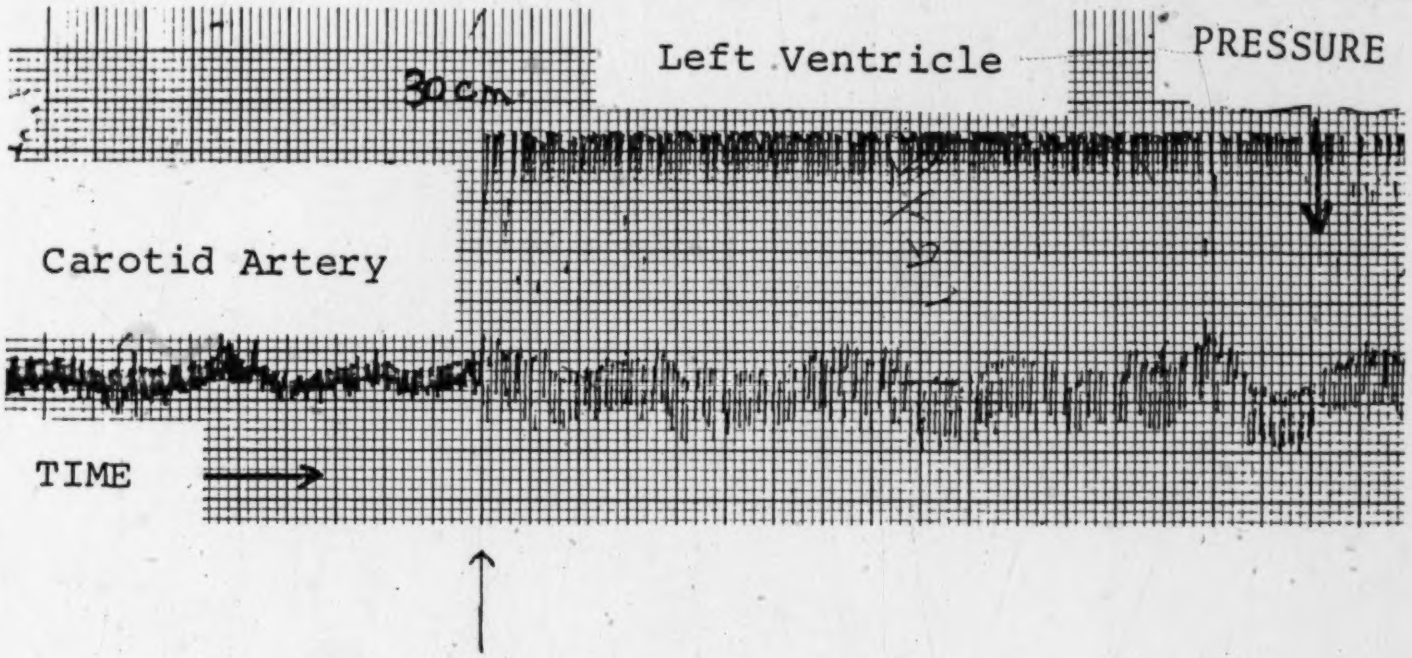
I. APPENDIX II :RESULTS (SUPPLEMENTARY)

The results sections of chapters 4 and 5 as well as the appendix data for individual animals contain a more complete account of the results obtained from this study. The purpose of the present appendix is to describe additional reasoning or methodology used to analyse the results. As with the supplementary methods in appendix I, the additional detail of the supplementary results was considered appropriate for thesis consideration.

II. 1 Catheter Placement

As was previously described in the review of literature, the radioactive microsphere technique, as used in the present experiment, requires the placement of a catheter into the left ventricle to facilitate the microsphere injection. Left ventricle catheter placement can be estimated by connecting the saline filled catheters to a pressure transducer in order to indicate the blood pressure changes in the various vessels and heart chamber sites. As the catheter is inserted down the carotid artery, the point at which the catheter enters the left ventricle is indicated by a drop in diastolic pressure. A trace of this characteristic is clearly illustrated in Figure 4.

Figure 4: Trace of systolic and dystolic blood pressure in the carotid artery and left ventricle as a 0.04 by 0.07 mm I.D. Tygon catheter was inserted down the left carotid artery of a sheep



Catheter tip entery into the left ventricle

However, due to the dynamics of the circulation system , such as heart movements and blood pressure changes, as well as the possibility of human error, the catheters can be subject to misplacement. Misplacement can occur either by improper original placement or by subsequent movement from their initial position. If misplacement by either circumstance occurs, the microspheres would be injected into the vascular system at a site which would not facilitate the distribution of microspheres to the forequarter areas of the body.

The verification of catheter placement upon dissection of the animal and analysis of tissue nuclide counts decidedly reflects catheter position at the time of microsphere injection. The misplacement of a catheter from the left ventricle can result in the injection of microspheres into one of two anatomical sites (1) the dorsal aorta, (2) the carotid artery.

In the present experiment, 2 of the 24 catheter placements were determined to be in a carotid artery and 8 of the 24 placements were determined to be in the descending aorta. The results from the carotid artery placements were rejected for use in this experiment, even though valuable information concerning blood flow via the carotid artery may be interpreted from these two sets of data. The results from the descending aorta injections were utilized in some treatment comparisons by comparing the relative distribution of microspheres to all tissues receiving blood via the descending aorta . These tissues included subcutaneous fat, spleen, pancreas, gall bladder, liver, adrenals, kidney, kidney fat, reticulo-rumen, omasum, abomasum, small intestine, large intestine, and urine bladder. Organs or tissues cephalic to the descending aorta flow were rejected.

An injection of microspheres into the descending aorta resulted in a greater number of spheres being distributed to the capillary beds of tissues receiving blood from the descending aorta; or, stated another way, this had the same effect as using a larger injection dose. To quantify how much larger the numbers of spheres were, an adjustment factor was calculated by comparing the GIT counts in "aorta injected" sheep versus "left ventricle injected" sheep within the same treatment group. An example of such a comparison would be as follows:

(1) Compare the total percent distribution of microspheres to the entire GIT (reticulo-rumen, omasum, abomasum, small intestine, large intestine) in the "aorta injected" sheep, to the treatment average percent distribution of microspheres to the entire GIT in the "left ventricle injected" sheep.

(2) Divide all percent distributions in the "aorta injected" sheep by the ratio.

specifically:

For the chronic cold treatment group (sheep#87, 78, 74, 80), the average GIT percent distribution of microspheres for the "left ventricle injected" sheep

=19.36%.

The Ce-141 injection in sheep #78 was determined to be into the descending aorta and the percent distribution to the GIT was 30.97%.

Therefore, the ratio is 30.97/19.36=1.6.

. Hence, percent distribution values for sheep 78 for the Ce-141 nuclide were divided by 1.6.

As a test of validity for this adjustment method, a statistical comparison of tissue counts obtained via "left ventricle" catheter placement versus "adjusted aorta" placements demonstrated no statistical differences within treatment (Table 8). Consequently, the adjusted values for the tissues and organs mentioned were used in subsequent treatment comparisons.

This same method of comparison could be used by comparing radioactive counts to any of several larger whole organs such as spleen, pancreas, or omasum between "left ventricle" and "aorta injected" sheep, and in fact similar results are obtained by doing so. However, by using the entire GIT, the adjustment factors are based on a larger pooled reference value and as such can be used with more assurance.

Table 8: Blood flow estimations (ml/100g/min) for sheep with microsphere injections in the left ventricle and descending aorta.

	Left Ventricle Placement				Adjusted Aorta	
	CON	AC	CC	SEM	CON	CC
Observations/mean	2	7	4		4	4
Internal Organs and Glands						
brain	63.9	83.1	113.8	13.8		
spinal cord	4.6	18.5	22.1	5.6		
heart	116.2	70.7	103.3	15.6		
lungs(bronch.art.)	57.5^{a}	5.9^{b}	12.2^{b}	2.9		
liver	2.7^{e}	2.2^{f}	8.2^{d}	4.2	9.8	4.2
kidney	225.4	338.9	193.7	36.5	168.7	173.9
thyroids	48.8^{e}	97.6^{d}	88.1^{e}	24.5		
pancreas	165.7	219.6	182.4	31.4	169.8	193.8
adrenals	84.5^{f}	202.4^{d}	140.1^{e}	37.7	177.9	76.2
gall bladder	7.7	9.1	5.7	1.2	13.9	8.5
urine bladder	5.6		6.5	3.1	15.7	5.2
spleen	78.8	120.1	92.5	26.6	63.6	67.2
Muscle						
longisimus dorsi	1.7	5.9	8.2	0.1		
gluteus maximus	3.0^{c}	5.6^{b}	14.3^{a}	1.3		
triceps	1.2^{f}	5.0^{e}	16.6^{d}	1.3		
diaphragm	28.6^{a}	11.3^{b}	28.2^{a}	2.6		
Gastrointestinal Tract						
esophagus	14.1	22.4	15.5	5.5		
reticulo-rumen	45.9^{d}	31.9^{e}	31.7^{f}	6.5	61.6	43.9
omasum	56.8	55.4	48.5	7.5	76.5	59.7
abomasum	103.5	137.6	70.0	16.7	80.4	76.5
small intestine	83.1	84.5	60.6	8.3	65.3	39.6
large intestine	43.2^{e}	67.6^{d}	51.1^{e}	8.7	73.3	55.2
Skin						
midside	5.6	12.0	7.8	1.2		
nasal	11.0	7.1	11.5	3.1		
lower front leg	1.1	1.1	0.9	0.6		
Fat						
subcutaneous	3.0	2.0	3.9	0.4	4.8	1.6
kidney fat	10.0	7.7	5.6	2.7	10.3	4.7
Bone						
radius and ulna	1.3^{d}	0.2^{f}	0.5^{e}	0.05		
nasal turbinals	12.3^{e}	4.2^{f}	19.7^{d}	5.2		
Other						
back hooves	1.0	0.5	0.4	0.05		
front hovves	0.8	0.5	0.4	0.05		
ears	2.3^{b}	2.7^{b}	8.5^{a}	3.2		

$^{a, b, c}$ Means with different superscripts within rows are statistically different at $P < 0.05$ and superscripts

$^{d, e, f}$ at $P < 0.10$ probability levels.

SEM is the standard error of treatment means.

Left ventricle placements refer to statistically adjusted means obtained via unequal numbers least squares analysis of variance.

Adjusted aorta values were obtained via the descending aorta adjustment proceedures as described in the text.

J. APPENDIX 3 :INDIVIDUAL ANIMAL DATA

The following appendix section contains values from individual animals for the various physiological measurements taken during the study. The sheep are listed according to treatment group starting with the control (CON) animals and followed by the acute cold (AC) and chronic cold (CC) animals. Within each treatment group, the animals are listed from lowest to higest identification number:

Control (CON) #8282, 8284, 8285, 8298

Acute cold (AC) #8265, 8270, 8271, 8279

Chronic cold (CC) #8274, 8278, 8280, 8287

Microsphere injections containing the radionuclides cerium-141 (Ce-141) and tin-113 (Sn-113) were carried out with measurements of heart rate and respiration rate made immediately befor the first injection, between injections, and after the second injection. The interval between injections was approximately 15-20 minutes. During the microsphere injections, blood samples were withdrawn from the right atria for calculations of arterial venous anastomosis (AVA). This blood sample was termed "syring right heart". Blood samples were also withdrawn from the carotid artery for the calculation of cardiac output. This blood sample was termed "syringe carotid artery". Blood sample withdrawal rates are shown in the appendix. Catheter placement into the left ventricle (LV), descending aorta

(DA) and carotid artery (CA) are also indicated in the following appendix tables.

APPENDIX

SHEEP 8282 CONTROL

Body weight (kg)	35.0	(34.0)*
Oxygen consumption (ml·min^{-1})	299.4	
Hematocrit (%)	x	
Hemoglobin (g·100 ml^{-1})	10.1	
Water intake (litter·day $^{-1}$)	2.9	
Digestibilities %		
Dry matter	54.1	
Protein	64.0	
Energy	54.5	
Acid detergent fiber	50.2	

Serum T_4 (µg·100 ml^{-1})	4.9
Skin Temperature (°C)	
Back	35.55
Flank	35.65
Midside	35.85
Lower Front Leg	33.88
Right Ear	33.35
Left Ear	33.70

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{113}Sn		^{141}Ce	
Heart rate (min^{-1})	96		120		114
Respiration rate (min^{-1})	55		52		40
Syringe right heart (ml·min^{-1})		14.8		15.6	
Syringe carotid artery (ml·min^{-1})		16.8		15.2	
Catheter Placement		DA		DA	

APPENDIX

SHEEP 8282 CONTROL

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	92489	1158161	44.9	13.2
reticulum	10149439	18126677	116.8	27.8
reticular mucosa**	1728	10967	8.9	0.7
rumen	26814175	54737419	469.9	101.7
rumen mucosa**	8752	25193	30.8	3.3
omasum	10150190	18916774	128.9	30.3
omasal mucosa**	1329	3322	23.9	3.0
abomasum	26196596	27369741	219.3	43.7
small intestine	40264139	61676483	592.4	122.3
large intestine	43010239	77172612	582.4	107.8
Internal Organs and Glands				
brain	18830	112000	88.0	16.5
spinal cord**	0	1741	5.9	0.7
heart	94250	59935	153.9	47.3
lungs	2249466	15519548	566.4	100.6
liver	4947378	8901419	601.3	149.5
kidney	8541694	39490709	101.8	19.7
thyroid	354	9258	6.3	1.0
pancreas	11963219	22709806	45.4	10.2
adrenal	1006675	3104322	8.1	1.3
gall bladder	80642	196516	6.4	0.8
urine bladder	63955	249645	16.2	1.8
spleen	11057053	13792580	231.8	70.0
Other Tissue and Organs				
Muscle				
longissimus dorsi**	1184831	1036870	42.6	10.6
gluteus maximus**	1279410	2690516	56.7	14.7
triceps**	3686	3258	44.7	11.4
diaphram	4355140	9747096	129.4	32.6
Skin				
midside**	359720	1003322	30.7	13.6
nasal**	5610	12774	43.6	13.7
lower front leg**	2196	5806	9.3	4.7
Fat				
subcutaneous**	215199	266612	26.8	9.8
kidney**	319299	1015870	35.9	7.4
Bone				
radius and ulna**	4586	12354	22.4	14.3
nasal turbinals	47340	60935	95.7	33.4
Other				
back hooves	74444	372354	73.9	39.0
front hooves	15482	12903	82.7	44.8
ears	5348	5516	36.2	14.4
Additional Measurements				
syringe right heart (Sn)	0	547580		
syringe right heart (Ce)	33824	0		
syringe carotid artery (Sn)	0	35000		
syringe carotid artery (Ce)	11085	0		
injectate	471194804	820092645		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8284 CONTROL

Body weight (kg)	40.0	(39.0)*
Oxygen consumption (ml·min^{-1})	347.9	
Hematocrit (%)	28.9	
Hemoglobin (g·100 ml^{-1})	9.8	
Water intake (litter·day^{-1})	3.0	
Digestibilities %		
Dry matter	54.4	
Protein	63.6	
Energy	54.7	
Acid detergent fiber	52.4	

Serum T_4 (μg·100 ml^{-1})	2.1
Skin Temperature (°C)	
Back	35.80
Flank	35.26
Midside	35.78
Lower Front Leg	33.70
Right Ear	32.84
Left Ear	31.10

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	150		140		x
Respiration rate (min^{-1})	126		87		x
Syringe right heart (ml·min^{-1})		12.6		x	
Syringe carotid artery (ml·min^{-1})		18.0		x	
Catheter Placement		LV			

APPENDIX

SHEEP 8284 CONTROL

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	549152	X	45.9	11.9
reticulum	4353217	X	146.4	27.8
reticular mucosa**	1086	X	X	X
rumen	22713586	X	763.5	159.0
rumen mucosa**	521	X	38.9	3.1
omasum	2460108	X	145.4	38.5
omasal mucosa**	1173	X	9.9	1.2
abomasum	11268130	X	229.3	48.1
small intestine	34762782	X	590.3	63.1
large intestine	16552978	X	579.3	98.1
Internal Organs and Glands				
brain	6498282	X	92.8	17.6
spinal cord**	18652	X	4.0	0.5
heart	24903956	X	176.3	52.6
lungs	1760043	X	534.1	105.9
liver	227847	X	673.1	194.6
kidney	38636782	X	148.2	28.7
thyroid	121434	X	6.3	1.3
pancreas	1658630	X	48.8	9.2
adrenal	514260	X	6.8	1.4
gall bladder	29434	X	13.6	1.4
urine bladder	57456	X	10.5	1.6
spleen	172239	X	49.8	11.1
Other Tissue and Organs				
Muscle				
longissimus dorsi**	3913	X	33.8	8.6
gluteus maximus**	21717	X	31.6	8.1
triceps**	27086	X	49.3	12.1
diaphragm	1986913	X	61.8	15.7
Skin				
midside**	75891	X	29.3	11.6
nasal**	548347	X	58.2	19.2
lower front leg**	8586	X	17.8	9.1
Fat				
subcutaneous**	1347	X	12.7	5.4
kidney**	22760	X	26.0	14.5
Bone				
radius and ulna**	23282	X	41.6	28.0
nasal turbinals	1461760	X	127.9	52.4
Other				
back hooves	36826	X	66.3	35.2
front hooves	56978	X	84.9	44.7
ears	65630		49.8	19.9
Additional Measurements				
syringe right heart (Sn)		X		
syringe right heart (Ce)	5869			
syringe carotid artery (Sn)		X		
syringe carotid artery (Ce)	1580869			
injectate	489056210			

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8285 CONTROL

Body weight (kg)	43.0	(44.0)*
Oxygen consumption ($ml\cdot min^{-1}$)	253.3	
Hematocrit (%)	28.5	
Hemoglobin ($g\cdot 100\ ml^{-1}$)	9.8	
Water intake ($litter\cdot day^{-1}$)	3.5	
Digestibilities %		
Dry matter	52.2	
Protein	62.2	
Energy	52.3	
Acid detergent fiber	50.2	

* pre treatment measurement

Serum T4 ($\mu g\cdot 100\ ml^{-1}$)	5.6
Skin Temperature (°C)	
Back	36.35
Flank	34.73
Midside	35.90
Lower Front Leg	34.18
Right Ear	33.75
Left Ear	32.93

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	144		140		108
Respiration rate (min^{-1})	72		80		52
Syringe right heart ($ml\cdot min^{-1}$)		x		x	
Syringe carotid artery ($ml\cdot min^{-1}$)		15.8		15.0	
Catheter Placement		DA		DA	

APPENDIX

SHEEP 8285 CONTROL

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	45058	167580	65.9	20.8
reticulum	7867943	8366548	144.5	28.3
reticular mucosa**	x	3612	1.1	0.1
rumen	30734722	44709354	581.1	126.7
rumen mucosa**	2126	6709	35.5	4.0
omasum	10602125	11161322	134.2	28.9
omasal mucosa**	141	5741	6.8	1.1
abomasum	17177439	16285935	222.6	44.0
small intestine	55720707	64337032	794.8	145.7
large intestine	25733134	29010580	336.8	65.1
Internal Organs and Glands				
brain	4880	71419	91.2	16.9
spinal cord**	921	6483	5.6	0.9
heart	65421	150774	208.3	58.8
lungs	1543764	5058387	684.1	131.1
liver	6531529	50295064*	495.2	135.5
kidney	9907814	36375258	108.0	22.4
thyroid	1629	9741	7.4	2.5
pancreas	2395423	5874677	49.8	12.7
adrenal	1663951	11173096*	8.3	1.3
gall bladder	371906	343258	25.3	2.1
urine bladder	235382	943096	20.5	4.7
spleen	12263816	23353161	181.6	53.4
Other Tissue and Organs				
Muscle				
longissimus dorsi**	477171	1050000	59.3	15.1
gluteus maximus**	503499	1385645	31.6	7.2
triceps**	1158	8096	34.7	1.0
diaphram	6688459	20276354	201.2	59.1
Skin				
midside**	210003	234064	18.1	7.4
nasal**	931.1	7580	46.6	14.7
lower front leg**	815.6	10387.1	22.3	9.2
Fat				
subcutaneous**	54751	31741	13.4	9.6
kidney**	365235	426645	42.9	12.9
Bone				
radius and ulna**	1397	5483	41.6	27.8
nasal turbinals	3871	13064	113.4	46.9
Other				
back hooves	198267	92225	65.2	35.4
front hooves	12227	2548	77.6	43.2
ears	87	5225	55.5	21.7
Additional Measurements				
syringe right heart (Sn)	0	0		
syringe right heart (Ce)	0	0		
syringe carotid artery (Sn)	0	37806		
syringe carotid artery (Ce)	3605	0		
injectate	533677304	797982645		

** Representative tissue sample, not whole organ or tissue

* liver and adrenal Sn counts for 8285 possibly high due to contamination

APPENDIX

SHEEP 8298 CONTROL

Body weight (kg)	44.0	(43.0)*
Oxygen consumption ($ml\cdot min^{-1}$)	364.1	
Hematocrit (%)	20.0	
Hemoglobin ($g\cdot 100\ ml^{-1}$)	7.5	
Water intake ($litter\cdot day^{-1}$)	3.5	
Digestibilities %		
Dry matter	55.5	
Protein	66.1	
Energy	55.7	
Acid detergent fiber	51.5	

Serum T4 ($\mu g.100\ ml^{-1}$)	6.6
Skin Temperature (°C)	
Back	34.38
Flank	34.86
Midside	35.86
Lower Front Leg	33.66
Right Ear	32.60
Left Ear	30.85

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{113}Sn		^{141}Ce	
Heart rate (min^{-1})	105		120		126
Respiration rate (min^{-1})	x		84		55
Syringe right heart ($ml\cdot min^{-1}$)		16.4		9.6	
Syringe carotid artery ($ml\cdot min^{-1}$)		18.8		11.4	
Catheter Placement		LV		CA	

APPENDIX

SHEEP 8298 CONTROL

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	0	1446354	69.8	16.5
reticulum	0	14356193	158.2	31.9
reticular mucosa**	2243	13483	5.9	0.3
rumen	0	45202161	566.5	114.6
rumen mucosa**	2939	15225	25.6	2.0
omasum	239017	15973580	119.5	23.4
omasal mucosa**	2032	5161	8.9	1.1
abomasum	1513999	49931709	233.2	49.1
small intestine	4604851	89734064	639.2	112.8
large intestine	760294	52648419	691.1	125.1
Internal Organs and Glands				
brain	17643079	6544806	95.9	18.4
spinal cord**	18338	36096	6.6	1.2
heart	228962	19267032	189.9	52.8
lungs	90284965	49576806	560.6	113.4
liver	1035723	6284322	869.9	191.6
kidney	9923852	34870935	159.9	25.3
thyroid	16835	674451	6.3	1.3
pancreas	1555671	22645903	54.6	12.0
adrenal	23490	986838	8.4	1.8
gall bladder	7835	158322	8.6	1.2
urine bladder	4680	90193	12.7	1.8
spleen	1304825	22610580	103.8	22.7
Other Tissue and Organs				
Muscle				
longissimus dorsi**	6251	178064	37.6	11.3
gluteus maximus**	8409	219000	29.6	7.1
triceps**	6810	139161	51.6	14.7
diaphram	84945	3652354	125.5	33.2
Skin				
midside**	1714	544774	46.2	19.0
nasal**	12400660	1232871	77.1	25.6
lower front leg**	629	26451	10.7	5.0
Fat				
subcutaneous**	2216	106516	12.7	6.7
kidney**	28293	1281387	47.6	14.7
Bone				
radius and ulna**	5625	77774	28.1	18.0
nasal turbinals	16089611	1601258	97.4	32.5
Other				
back hooves	19	128903	66.9	33.1
front hooves	5141	108612	90.2	46.8
ears	4912925	259580	57.9	21.2
Additional Measurements				
syringe right heart (Sn)	0	114677		
syringe right heart (Ce)	130952	0		
syringe carotid artery (Sn)	0	1390838		
syringe carotid artery (Ce)	29126			
injectate	612705260	788749250		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8265 ACUTE COLD

Body weight (kg)	36.0	(41.0)*	Arterial blood pO_2 (mm Hg)	78.6
Oxygen consumption ($ml \cdot min^{-1}$)	407.4	(252.2)*	Venous blood pO_2 (mm Hg)	42.5
Hematocrit (%)	35.5		Arterial blood pCO_2 (mm Hg)	58.2
Hemoglobin ($g \cdot 100\ ml^{-1}$)	10.3		Venous blood pCO_2 (mm Hg)	69.8
Blood pH (arterial)	7.17		Venous blood pH	6.99

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{113}Sn		^{141}Ce	
Heart rate (min^{-1})	132		120		105
Respiration rate (min^{-1})	20		24		18
Syringe right heart ($ml \cdot min^{-1}$)		X		5.0	
Syringe carotid artery ($ml \cdot min^{-1}$)		19.0		19.0	
Catheter Placement		LV		LV	

APPENDIX

SHEEP 8265 ACUTE COLD

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	833576	1701106	46.8	13.4
reticulum	3783444	4622878	88.7	20.2
reticular mucosa**	Y	Y	Y	Y
rumen	9881621	12885485	439.2	96.7
rumen mucosa**	Y	Y	Y	Y
omasum	3873795	5139601	97.9	18.7
omasal mucosa**	Y	Y	Y	Y
abomasum	11271313	22011704	110.5	28.5
small intestine	40453161	40192319	491.9	54.3
large intestine	24370537	29749714	410.0	109.3
Internal Organs and Glands				
brain	4971095	5315601	86.5	16.8
spinal cord**	22900	27080	0.7	X
heart	13169254	11159930	159.7	75.3
lungs	4379699	6395008	619.8	104.6
liver	638716	1099375	770.3	174.9
kidney	25002368	36400219	106.3	21.9
thyroid	330110	425476	6.0	1.3
pancreas	X	X	X	X
adrenal	627312	782557	5.0	0.8
gall bladder	104020	173270	12.3	2.8
urine bladder	X	X	X	X
spleen	3632944	6551594	37.7	8.6
Other Tissue and Organs				
Muscle				
longissimus dorsi**	187131	82511	17.9	5.4
gluteus maximus**	275182	135840	28.9	8.0
triceps**	784306	405082	69.0	18.9
diaphram	556385	643709	65.0	18.9
Skin				
midside**	95042	141759	18.3	6.4
nasal**	304338	411724	44.0	16.3
lower front leg**	76098	25630	15.6	6.8
Fat				
subcutaneous**	14613	12566	18.1	17.7
kidney**	462340	73768	41.6	32.9
Bone				
radius and ulna**	13426	10216	27.7	23.8
nasal turbinals	814617	1032832	173.3	74.8
Other				
back hooves	72965	28302	103.9	17.5
front hooves	61574	99200	103.0	17.5
ears	80972	72850	49.5	36.0
Additional Measurements				
syringe right heart (Sn)	X	X		
syringe right heart (Ce)	398	0		
syringe carotid artery (Sn)	0	2066710		
syringe carotid artery (Ce)	1836490	0		
injectate	646763387	671853032		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8270 ACUTE COLD

Body weight (kg)	49.0	(52.0)*	Arterial blood pO_2 (mm Hg)	81.9
Oxygen consumption ($ml \cdot min^{-1}$)	502.0	(292.5)*	Venous blood pO_2 (mm Hg)	28.8
Hematocrit (%)	30.8		Arterial blood pCO_2 (mm Hg)	70.4
Hemoglobin ($g \cdot 100\ ml^{-1}$)	7.1		Venous blood pCO_2 (mm Hg)	73.5
Blood pH (arterial)	7.15		Venous blood pH	7.21

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	96		128		108
Respiration rate (min^{-1})	30		21		21
Syringe right heart ($ml \cdot min^{-1}$)		18.5		17.0	
Syringe carotid artery ($ml \cdot min^{-1}$)		19.5		19.0	
Catheter Placement		LV		LV	

APPENDIX

SHEEP 8270 ACUTF . COLD

	DPM ^{141}Ce	DPM ^{113}Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	1174565	762757	72.8	41.4
reticulum	6051039	7642776	124.7	29.5
reticular mucosa**	Y	Y	Y	Y
rumen	18300014	20241447	638.4	103.1
rumen mucosa**	Y	Y	Y	Y
omasum	6889354	10321189	159.6	32.5
omasal mucosa**	Y	Y	Y	Y
abomasum	15836722	23796580	176.8	45.5
small intestine	42195121	61878269	765.4	97.0
large intestine	22114057	31817881	497.6	112.0
Internal Organs and Glands				
brain	3957819	6327408	104.5	X
spinal cord**	6075	13168	7.5	X
heart	7209978	10593266	223.2	77.1
lungs	4543048	4010502	782.2	142.8
liver	725014	1380008	801.4	156.1
kidney	28252641	42515335	147.8	28.1
thyroid	271236	669448	6.9	1.5
pancreas	2942425	5102947	21.8	7.1
adrenal **	172748	400999	0.9	0.6
gall bladder	46980	133457	13.6	1.5
urine bladder	X	X	X	X
spleen	5219691	19757804	98.5	25.4
Other Tissue and Organs				
Muscle				
longissimus dorsi**	125891	188044	131.8	39.8
gluteus maximus**	110620	174061	56.5	20.9
triceps**	63739	162884	73.5	X
diaphram	821723	1104144	176.6	49.8
Skin				
midside**	222159	308391	28.9	13.1
nasal**	391478	358773	69.5	X
lower front leg**	10331	13279	27.7	11.8
Fat				
subcutaneous**	14281	16558	25.8	23.8
kidney**	198790	244262	91.0	81.8
Bone				
radius and ulna**	2723	3507	30.5	28.4
nasal turbinals	332090	451532	116.8	45.1
Other				
back hooves	26441	31295	68.4	41.0
front hooves	24092	20742	87.2	46.0
ears	112153	80620	50.9	31.5
Additional Measurements				
syringe right heart (Sn)	0	4653		
syringe right heart (Ce)	6807	0		
syringe carotid artery (Sn)	0	1310092		
syringe carotid artery (Ce)	784146	0		
injectate	362423167	518686115		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8271 ACUTE COLD

Body weight (kg)	48.0	(52.0)*	Arterial blood pO_2 (mm Hg)	74.5
Oxygen consumption ($ml \cdot min^{-1}$)	364.8	(302.8)*	Venous blood pO_2 (mm Hg)	36.1
Hematocrit (%)	27.8		Arterial blood pCO_2 (mm Hg)	62.3
Hemoglobin ($g \cdot 100\ ml^{-1}$)	8.3		Venous blood pCO_2 (mm Hg)	104.0
Blood pH (arterial)	7.19		Venous blood pH	6.98

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	150		120		132
Respiration rate (min^{-1})	20		18		24
Syringe right heart ($ml \cdot min^{-1}$)		17.0		10.0	
Syringe carotid artery ($ml \cdot min^{-1}$)		x		15.0	
Catheter placement		LV		CA	

APPENDIX

SHEEP 8271 ACUTE COLD

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	823148	1237	36.9	11.7
reticulum	3496389	4043	187.5	36.9
reticular mucosa**	Y	Y	Y	Y
rumen	9642854	13028	670.7	138.5
rumen mucosa**	Y	Y	Y	Y
omasum	4170557	3284	133.9	27.4
omasal mucosa**	Y	Y	Y	Y
abomasum	31381553	27129	296.8	53.1
small intestine	43769619	44695	584.7	68.6
large intestine	25062871	29587	452.7	80.7
Internal Organs and Glands				
brain	7992558	1876515	104.4	21.5
spinal cord**	16378	49	X	X
heart	20184820	19363	254.4	87.3
lungs	1936834	11096321	808.1	111.4
liver	1372556	54311	940.6	235.5
kidney	44355910	86947	125.1	26.1
thyroid	505217	322	7.3	1.6
pancreas	5557533	5870	44.8	9.5
adrenal	382238	495	5.8	0.8
gall bladder	155484	310	17.7	5.1
urine bladder	X	X	X	X
spleen	6173386	9252	104.9	22.7
Other Tissue and Organs				
Muscle				
longissimus dorsi**	646195	654	68.8	19.8
gluteus maximus**	277868	327	77.9	23.0
triceps**	306455	327	111.4	30.7
diaphram	1513600	3230	134.1	41.4
Skin				
midside**	275086	667	17.0	5.9
nasal**	313966	3647345	58.7	20.1
lower front leg**	7406	1856	20.2	8.5
Fat				
subcutaneous**	157315	217	40.5	23.9
kidney**	1215349	2168	126.1	53.6
Bone				
radius and ulna**	3232	3367	32.6	26.3
nasal turbinals	230171	14987036	170.3	74.4
Other				
back hooves	54644	2474	96.2	54.6
front hooves	18226	1746	96.0	48.6
ears	52837	1393405	64.2	25.4
Additional Measurements				
syringe right heart (Sn)	0	13223		
syringe right heart (Ce)	1645	0		
syringe carotid artery (Sn)	0	16906		
syringe carotid artery (Ce)	0	0		
injectate	470834017	469650947		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8279 ACUTE COLD

Body weight (kg)	46.0	(53.0)*	Arterial blood pO_2 (mm Hg)	74.2
Oxygen consumption ($ml \cdot min^{-1}$)	588.4	(285.6)*	Venous blood pO_2 (mm Hg)	32.5
Hematocrit (%)	35.0		Arterial blood pCO_2 (mm Hg)	54.2
Hemoglobin ($g \cdot 100\ ml^{-1}$)	7.1		Venous blood pCO_2 (mm Hg)	81.8
Blood pH (arterial)	7.12		Venous blood pH	6.88

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	113		102		126
Respiration rate (min^{-1})	20		20		24
Syringe right heart ($ml \cdot min^{-1}$)		18.5		19.0	
Syringe carotid artery ($ml \cdot min^{-1}$)		14.0		19.0	
Catheter Placement		LV		LV	

APPENDIX

SHEEP 8279 ACUTE COLD

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	313915	222667	25.1	9.0
reticulum	2884000	2469411	117.0	25.3
reticular mucosa**	Y	Y	Y	Y
rumen	12656137	8906126	573.7	143.1
rumen mucosa**	Y	Y	Y	Y
omasum	8724936	5548406	139.8	26.5
omasal mucosa**	Y	Y	Y	Y
abomasum	19267970	12656548	215.7	54.9
small intestine	33718640	27222713	618.8	85.4
large intestine	19468049	18098538	427.3	75.9
Internal Organs and Glands				
brain	12194394	12958236	106.7	20.1
spinal cord**	41902	61625	X	X
heart	7732113	8167907	189.0	61.4
lungs	3344587	2803634	688.0	112.7
liver	874106	835974	886.3	210.1
kidney	32133510	30531217	139.8	27.9
thyroid	1742708	1685488	10.6	2.3
pancreas	9392732	8653339	45.5	10.7
adrenal	340077	360874	2.2	0.7
gall bladder	69908	48085	53.3	3.5
urine bladder	X	X	X	X
spleen	3420838	2998009	98.2	36.3
Other Tissue and Organs				
Muscle				
longissimus dorsi**	439456	178115	83.9	24.8
gluteus maximus**	439732	594077	59.7	17.7
triceps**	471362	966923	92.5	27.2
diaphram	1546848	1738173	105.5	36.3
Skin				
midside**	78044	70945	16.8	5.5
nasal**	424331	321385	49.8	18.9
lower front leg**	4230	3267	15.7	7.9
Fat				
subcutaneous**	18887	43173	16.6	X
kidney**	266504	1180576	71.9	66.2
Bone				
radius and ulna**	2378	2604	24.6	21.9
nasal turbinals	1178372	935482	133.9	61.6
Other				
back hooves	12901	17402	86.8	50.4
front hooves	27464	37624	76.2	43.6
ears	173382	243014	56.8	35.1
Additional Measurements				
syringe right heart (Sn)	0	6663		
syringe right heart (Ce)	4419	0		
syringe carotid artery (Sn)	0	2893706		
syringe carotid artery (Ce)	1843126	0		
injectate	663555516	722767767		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8274 CHRONIC COLD

Body weight (kg)	34.0	(42.0)*
Oxygen consumption (ml·min^{-1})	456.9	
Hematocrit (%)	30.0	
Hemoglobin (g·100 ml^{-1})	10.2	
Water intake (litter·day^{-1})	3.1	
Digestibilities %		
Dry matter	52.2	
Protein	63.1	
Energy	53.2	
Acid detergent fiber	49.6	

Serum T4 (μg·100 ml^{-1})	9.1
Temperature (°C)	
Back	30.75
Flank	33.53
Midside	33.23
Lower Front Leg	23.30
Right Ear	19.66
Left Ear	18.81

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	135		112		102
Respiration rate (min^{-1})	24		26		28
Syringe right heart (ml·min^{-1})		3.8		7.2	
Syringe carotid artery (ml·min^{-1})		15.4		16.8	
Catheter Placement		LV		LV	

APPENDIX

SHEEP 8274 CHRONIC COLD

	DPM ^{141}Ce	DPM ^{113}Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	284017	828419	29.0	6.8
reticulum	1912836	9216290	111.2	16.1
reticular mucosa**	538	1774	5.8	0.3
rumen	5512669	25489419	533.6	90.6
rumen mucosa**	589	1871	13.9	1.2
omasum	2427647	9080967	117.9	17.9
omasal mucosa**	1048	1419	4.9	0.1
abomasum	12423289	37482225	307.0	51.5
small intestine	20557498	76956129	878.4	132.5
large intestine	11156616	40556032	374.9	51.0
Internal Organs and Glands				
brain	3606276	13749096	92.9	18.2
spinal cord**	159538	659871	9.2	1.5
heart	10470127	34707516	173.8	37.7
lungs	2038373	12107354	494.2	92.7
liver	903201	39909580	930.8	224.7
kidney	7500695	44904387	138.9	26.4
thyroid	114158	571161	7.4	0.9
pancreas	2186396	12374709	36.3	7.0
adrenal	564060	3242838	7.9	0.9
gall bladder	40546	244677	17.4	1.3
urine bladder	53256	488258	24.2	3.5
spleen	1561926	18619935	70.3	15.2
Other Tissue and Organs				
Muscle				
longissimus dorsi**	268582	356903	19.8	4.1
gluteus maximus**	275412	529064	19.8	4.3
triceps**	410645	629225	15.8	2.8
diaphram	3624039	8745451	182.8	40.4
Skin				
midside**	87155	261677	17.5	5.8
nasal**	27108	110000	27.2	7.8
lower front leg**	0	4838	22.0	9.7
Fat				
subcutaneous**	40054	54419	7.8	3.2
kidney**	9525	87451	5.8	0.9
Bone				
radius and ulna**	2453	27483	40.9	26.4
nasal turbinals	515523	3302516	168.2	58.5
Other				
back hooves	15109	35161	64.1	31.7
front hooves	10631	38935	78.6	40.1
ears	479505	2667354	66.1	21.6
Additional Measurements				
syringe right heart (Sn)	0	56129		
syringe right heart (Ce)	4022	0		
syringe carotid artery (Sn)	0	2562225		
syringe carotid artery (Ce)	1589740	0		
injectate	401279456	838557451		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8278 CHRONIC COLD

Body weight (kg)	36.0	(44.0)*
Oxygen consumption ($ml\cdot min^{-1}$)	366.6	
Hematocrit (%)	20.0	
Hemoglobin ($g\cdot 100\ ml^{-1}$)	7.6	
Water intake ($litter\cdot day^{-1}$)	2.4	
Digestibilities %		
Dry matter	52.1	
Protein	63.6	
Energy	52.3	
Acid detergent fiber	50.0	

* pre treatment measurement

Serum T_4 ($\mu g\cdot 100\ ml^{-1}$)	6.5
Temperature (^{o}C)	
Back	29.48
Flank	30.88
Midside	31.08
Lower Front Leg	24.18
Right Ear	15.88
Left Ear	15.15

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{113}Sn		^{141}Ce	
Heart rate (min^{-1})	190		197		145
Respiration rate (min^{-1})	17		18		23
Syringe right heart ($ml\cdot min^{-1}$)		16.0		14.8	
Syringe carotid artery ($ml\cdot min^{-1}$)		16.4		15.2	
Catheter Placement		DA		DA	

APPENDIX

SHEEP 8278 CHRONIC COLD

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	894	3612	32.2	8.9
reticulum	12278620	17982193	150.0	28.9
reticular mucosa**	413	2612	3.6	0.1
rumen	38587999	59334645	544.7	124.3
rumen mucosa**	4459	18871	30.8	4.8
omasum	11326554	16060225	132.5	23.4
omasal mucosa**	186	2096	4.6	0.4
abomasum	27089462	56348258	185.6	39.4
small intestine	65607677	109057225	990.4	210.7
large intestine	36093843	53349193	430.1	81.0
Internal Organs and Glands				
brain	266	2871	97.3	18.6
spinal cord**	459	2741	4.6	0.9
heart	12204	39612	184.7	52.1
lungs	119411	1346419	43.9	7.4
liver	3159810	5638580	919.3	210.2
kidney	27877883	81559774	149.7	28.4
thyroid	26	2290	6.3	1.8
pancreas	11621519	22710709	38.9	7.6
adrenal	501310	1087000	5.8	1.0
gall bladder	122961	226064	7.5	0.9
urine bladder	94036	275838	22.0	3.3
spleen	18161935	31732483	139.4	39.3
Other Tissue and Organs				
Muscle				
longissimus dorsi**	163130	143709	11.6	2.7
gluteus maximus**	343062	623387	26.9	6.4
triceps**	403	2677	29.5	7.1
diaphram	300584	1113354	66.9	18.7
Skin				
midside**	173360	373354	29.6	9.1
nasal**	1099	4129	39.7	13.7
lower front leg**	1058	3677	38.5	17.2
Fat				
subcutaneous**	9670	23129	21.6	12.5
kidney**	40904	233129	11.1	7.5
Bone				
radius and ulna**	1189	4258	42.4	28.5
nasal turbinals	6316	19096	112.0	48.6
Other				
back hooves	25394	69290	56.5	28.0
front hooves	2411	4516	74.8	37.8
ears	3466	4290	53.3	19.5
Additional Measurements				
syringe right heart (Sn)	0	51871		
syringe right heart (Ce)	7667	0		
syringe carotid artery (Sn)	0	31419		
syringe carotid artery (Ce)	3863	0		
injectate	616912456	825033095		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8280 CHRONIC COLD

Body weight (kg)	43.0	(52.0)*
Oxygen consumption ($ml \cdot min^{-1}$)	422.4	
Hematocrit (%)	x	
Hemoglobin ($g \cdot 100\ ml^{-1}$)	8.1	
Water intake ($litter \cdot day^{-1}$)	2.6	
Digestibilities %		
Dry matter	54.6	
Protein	65.2	
Energy	54.7	
Acid detergent fiber	52.5	

Serum T_4 ($\mu g \cdot 100\ ml^{-1}$)	9.8
Temperature (°C)	
Back	29.85
Flank	32.80
Midside	32.25
Lower Front Leg	24.66
Right Ear	20.88
Left Ear	21.35

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{141}Ce		^{113}Sn	
Heart rate (min^{-1})	210		180		180
Respiration rate (min^{-1})	20		20		18
Syringe right heart ($ml \cdot min^{-1}$)		15.0		15.0	
Syringe carotid artery ($ml \cdot min^{-1}$)		6.2		3.6	
Catheter Placement		DA		DA	

APPENDIX

SHEEP 8280 CHRONIC COLD

	DPM 141 Ce	DPM 113 Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	248012	644645	55.2	16.2
reticulum	13433746	24354354	192.6	41.4
reticular mucosa**	3251	2967	5.6	0.5
rumen	29344072	49886903	529.2	110.2
rumen mucosa**	507206	1234161	19.9	2.5
omasum	9118388	17274064	100.8	20.4
omasal mucosa**	5984	6935	6.8	0.6
abomasum	7576987	36805806	204.2	43.3
small intestine	50664265	88305258	993.0	197.4
large intestine	37454759	82072225	511.7	101.1
Internal Organs and Glands				
brain	819	2806	111.0	21.2
spinal cord**	99067	239741	7.8	1.4
heart	20024	31516	251.7	72.1
lungs	760232	5374064	729.6	118.6
liver	4548261	21261548	967.8	244.4
kidney	30222928	79569064	148.5	27.4
thyroid	1205	1677	6.8	1.4
pancreas	17871582	26387419	66.9	12.4
adrenal	785905	1560064	6.9	0.9
gall bladder	91057	241838	13.6	1.5
urine bladder	225667	571096	30.3	4.6
spleen	5739008	31704677	167.7	49.2
Other Tissue and Organs				
Muscle				
longissimus dorsi**	1042035	879741	56.4	16.9
gluteus maximus**	3850706	3123580	50.8	12.7
triceps**	2007	2419	46.9	11.7
diaphram	1166644	1850677	135.1	43.5
Skin				
midside**	112196	264161	15.6	4.7
nasal**	778	4096	61.9	20.8
lower front leg**	287	2580	35.5	13.3
Fat				
subcutaneous**	66259	149677	19.6	8.8
kidney**	175136	557645	42.4	17.6
Bone				
radius and ulna**	1531	0	45.0	30.8
nasal turbinals	5394	23806	101.0	36.4
Other				
back hooves	11532	38870	64.1	31.6
front hooves	1606	1612	81.1	41.7
ears	3268	2129	29.7	10.4
Additional Measurements				
syringe right heart (Sn)	0	36903		
syringe right heart (Ce)	4559	0		
syringe carotid artery (Sn)	0	4032		
syringe carotid artery (Ce)	3450	0		
injectate	786418150	869167090		

** Representative tissue sample, not whole organ or tissue.

APPENDIX

SHEEP 8287 CHRONIC COLD

Body weight (kg)	46.0	(57.0)*
Oxygen consumption ($ml \cdot min^{-1}$)	345.5	
Hematocrit (%)	25.8	
Hemoglobin ($g \cdot 100\ ml^{-1}$)	8.9	
Water intake ($litter \cdot day^{-1}$)	2.8	
Digestibilities %		
Dry matter	52.6	
Protein	62.9	
Energy	53.4	
Acid detergent fiber	48.4	

Serum T_4 ($\mu g \cdot 100\ ml^{-1}$)	4.0
Temperature (°C)	
Back	29.68
Flank	30.26
Midside	30.96
Lower Front Leg	23.33
Right Ear	19.86
Left Ear	19.72

* pre treatment measurement

MICROSPHERE INJECTION

	Before first		Between injections		After second
Injection Order		^{113}Sn		^{141}Ce	
Heart rate (min^{-1})	132		150		150
Respiration rate (min^{-1})	23		29		22
Syringe right heart ($ml \cdot min^{-1}$)		17.6		16.2	
Syringe carotid artery ($ml \cdot min^{-1}$)		18.4		17.2	
Catheter Placement		LV		LV	

APPENDIX

SHEEP 8287 CHRONIC COLD

	DPM ^{141}Ce	DPM ^{113}Sn	Wet weight (g)	Dry weight (g)
Gastrointestinal Tract				
esophagus	540218	908451	46.8	12.0
reticulum	4520230	8085645	130.4	24.1
reticular mucosa**	0	1516	6.3	0.4
rumen	13075709	24559483	541.8	121.7
rumen mucosa**	1969	6161	51.9	7.4
omasum	5613503	10200645	137.8	30.5
omasal mucosa**	628	4806	9.4	1.0
abomasum	18169762	35701967	355.7	72.5
small intestine	33636847	57823451	613.8	87.0
large intestine	23556591	38546902	654.0	105.1
Internal Organs and Glands				
brain	10739639	17785322	97.6	18.5
spinal cord**	70638	104419	8.0	1.5
heart	24165908	37004967	304.3	90.0
lungs	4832011	12234741	633.6	115.7
liver	838974	3086774	952.6	240.2
kidney	29523797	46793709	165.5	32.2
thyroid	599221	1605032	7.6	1.7
pancreas	9317879	21148290	70.2	6.7
adrenal	743872	1357580	10.2	2.1
gall bladder	85600	183870	24.6	1.6
urine bladder	91691	167709	25.8	5.4
spleen	4040293	11828903	89.3	20.1
Other Tissue and Organs				
Muscle				
longissimus dorsi**	42657	99064	18.9	4.5
gluteus maximus**	332100	847483	45.4	12.0
triceps**	104003	246419	23.8	5.4
diaphram	2944371	5191354	141.5	38.2
Skin				
midside**	196137	337354	34.9	10.5
nasal**	806863	669903	43.9	14.2
lower front leg**	29442	68967	44.7	15.8
Fat				
subcutaneous**	73254	85774	29.8	15.8
kidney**	137482	173903	32.9	15.4
Bone				
radius and ulna**	20035	44225	49.0	32.5
nasal turbinals	4847594	3953161	176.5	67.3
Other				
back hooves	23868	54345	73.9	36.7
front hooves	33478	62774	94.9	48.3
ears	3022	7838	26.9	8.9
Additional Measurements				
syringe right heart (Sn)	0	40161		
syringe right heart (Ce)	10230	0		
syringe carotid artery (Sn)	0	2475870		
syringe carotid artery (Ce)	1022557	0		
injectate	523948326	816559677		

** Representative tissue sample, not whole organ or tissue.